SCIENCE

JULY 27, 1951

VOLUME 114 NUMBER 2952

Contents

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

the new *Lab-aid* way to store and view color transparencies

Assembly of Lab-aid Transparency Tray Cabinets, with the illuminator cabinet drawer pulled out. Use the table base, or stack them with your present Lab-aid Files.

You know how efficiently Technicon Lab-aid cabinets bring order to the filing of microslides, paraffin blocks and associated materials in the pathologic laboratory.

Now, in this new addition to the Lab-aid Filing System, you can enjoy the same benefits in the filing and viewing of color transparencies and lantern slides. The new Lab-aid transparency file has 40 smooth-sliding flat trays, each holding 16 standard 2" mounts, or 5 3¼" x 4¼" lantern slides in full view. They're easy to insert . . . you just drop them flat in the grooved frames. Easy to remove, too, by popping them out from underneath.

The special cabinet with its pull-out illuminator drawer lets you view a whole tray of transparencies at a glance, without removing any of them. Sitting or standing, you can inspect hundreds of slides under ideal viewing conditions without stirring from your place. And the disappearing shelf is a mighty handy place to lay slides down temporarily while you're working with them.

Both tray and illuminator cabinets are of the same dimensions and finish as all the other units in the Technicon Laboratory Filing System, and can be stacked interchangeably with them. If you would like more information about these or other units of the system, we'll be glad to send details.

The disappearing shelf is convenient as a temporary resting place for slides while working with them.

Inspect all your slides without removing any of them, or even stirring from your place.

Other units in the Lab-aid line

1" MICROSLIDE DRAWER FILES
2" SLIDE OR TRANSPARENCY DRAWER FILE
4" SLIDE OR INDEX DRAWER FILES
MICROSLIDE FLAT-FILING TRAY CABINET
PARAFFIN BLOCK FILE

technicon *Lab-aid* filing system

THE TECHNICON COMPANY
215 East 149th St., New York 51, N. Y.

Mathematical Biology

THE aim of mathematical biology is to introduce into the biological sciences not only quantitative, but also deductive, methods of research. The underlying idea has been to apply to biology the method by which mathematics has been successfully utilized in the physical sciences. This method can be briefly described.

First, the actual situation, the biological problem presented by nature, is replaced by an idealized model. This is done because one cannot hope to deal with all aspects of reality at once, and also because some of these aspects may be irrelevant to the question at hand. Next, the idealized model is stated in mathematical terms, and the consequences of the mathematical statement are derived. Finally, these deduced results must be reinterpreted in terms of the original biological problem.

In its early development mathematical biology was largely confined to mathematical genetics and ecology, as represented by the work of R. A. Fisher, J. B. S. Haldane, S. Wright, V. Volterra, V. A. Kostitzin, A. Lotka, and G. F. Gause. With the organization of the Committee on Mathematical Biology (formerly Section of Mathematical Biophysics) at the University of Chicago under N. Rashevsky in 1934, a group of workers, devoting themselves exclusively to this method, have developed mathematical theories of a number of diversified biological phenomena. Among these may be mentioned the following:

Mathematical biophysics of metabolizing systems. The applications of diffusion equations to idealized models of cells characterized by specific metabolic processes and semipermeable membranes lead to equations relating rates of respiration to oxygen concentrations, mathematical expressions describing the deformation of cells during their division, predictions concerning critical size of cells, and rates of growth and division as functions of biochemical parameters, such as the glycolytic coefficient.

The theory of nervous excitation. On the basis of certain assumptions governing the rate of accumulation and dissipation of "excitatory" and "inhibitory" effects impinging on a nerve fiber, equations are derived relating excitation time to the strength of impinging stimulus, the magnitude of an alternating current just sufficient to excite to the frequency, velocity of impulse propagation to the diameter of the fiber, etc. Recently the phenomenon of nerve excitation, particularly its "all-or-none" character, was theoretically related to electrochemical events in the vicinity of the nerve cell.

The theory of transynaptic transmission of excitation. Various models of the central nervous system were constructed to account for conditioning, learning, discrimination, and abstracting phenomena. The derived equations concern such matters as reaction times under various conditions, memory curves, learning curves, discrimination accuracy, and other psychophysical data.

To mention other fields of investigation in mathematical biology, one might list theories on the biological effects of radiation, theories of ontogenetic and phylogenetic development of organic form, and the application of thermodynamic principles to theories of physiological equilibria.

Recent trends indicate a concentration on stochastic methods, in particular their application in attempts to construct a "statistical dynamics" of the nervous system, viewed as a vast collection of units (neurons) interacting in accordance with certain laws. Suggestions implicit in the theory of servomechanisms (cybernetics) are also being investigated for their possible applications to the theory of the nervous system, viewed as a communication mechanism in which closed loop circuits play a prominent part.

It is hoped that in time the suggestions of Schroedinger and Jordan along the lines of applying quantum-mechanical principles to the theory of the life processes will likewise find fruitful application.

ANATOL RAPOPORT
H. G. LANDAU

Committee on Mathematical Biology
University of Chicago

SCIENCE, founded in 1880, is published each Friday by the American Association for the Advancement of Science at the Business Press, 10 McGovern Ave., Lancaster, Pa. Entered as second-class matter at the Post Office at Lancaster, Pa., January 13, 1948, under the Act of March 3, 1879. Acceptance for mailing at the special rate postage provided for in the Act of February 28, 1925, embodied in Paragraph (d-2) Section 34.40 P. L. & R. of 1948.

All correspondence should be sent to SCIENCE, 1515 Massachusetts Ave., N.W., Washington 5, D. C. The AAAS assumes no responsibility for the safety of manuscripts or for the opinions expressed by contributors. Four weeks' notice is required for change of address, and an address stencil label from a recent issue must be furnished. Claims for a missing number will be allowed only if received within 60 days from date of issue.

Annual subscriptions, $7.50; single copies, $.25; foreign postage, outside the Pan-American Union, $1.00; Canadian postage, $.50. Special rates to members of the AAAS.

The AAAS also publishes THE SCIENTIFIC MONTHLY. Subscription and advertising rates on request.

Publications Received

Research and Related Services in the United States Department of Agriculture. Repr. Pt. I. Prepared for the Committee on Agriculture of the House of Representatives, 81st Congress, Second Session. GPO, Washington, D. C. 1951.

Report of the Executive Committee 1950. F. J. M. Stratton, Ed. International Council of Scientific Unions. University Press, Cambridge, Eng. 5s.

1949–50 Annual Report of the Statistical Laboratory of Iowa State College. Bureau of Agricultural Economics, Ames.

Statistics of Nonpublic Secondary Schools 1947–48. Prepared by Rose Marie Smith. FSA, Washington, D. C. 1951. 10¢.

Alameda County Mosquito Abatement District. Annual Report 1950. Alameda County Mosquito Abatement District, Oakland, Calif. 1951.

Archeology of the Bynum Mounds, Mississippi. John L. Cotter and John M. Corbett. GPO, Washington, D. C. 1951. 75¢.

Consulting Services. 13th ed. Association of Consulting Chemists and Chemical Engineers, Inc., New York. 1951.

Contributions to the Metamorphosis and Biology of the Genus Atrichopogon Kieffer (Diptera, Ceratopogonidæ). Anker Nielsen. 1951. $2.75. ***Cytological and Embryological Studies in the Amphi-Apomictic Arabis Holboellii Complex.*** Tyge W. Böcher. Ejnar Munksgaard, Copenhagen. 1951. $1.75.

Data Sheets on Matheson Gas Data Book. Matheson Co., East Rutherford, N. J. 1951. $2.00.

Guia de Instituciones y Sociedades Cientificas Latinoamericanas, Cuarta Parte, ***Cuba, República Dominicana y Haiti.*** Union Panamericana, Washington, D. C. 1951. 15 cents.

Improving School Holding Power. Circ. 291. GPO, Washington, D. C. 1951. 40¢.

Problems of Cosmical Aerodynamics. Proc. Symposium on Motion of Gaseous Masses of Cosmical Dimensions Held at Paris, Aug. 16–19, 1949. Central Air Documents Office (Army-Navy-Air Force), Dayton, Ohio. 1951.

The Psychological Corporation, 29th Annual Report. Psychological Corporation, New York. 1950.

Quiroga, A Mexican Municipio. Donald D. Brand. GPO, Washington, D. C. 1951. $1.75.

Second Annual Report of the Division of Education. Carnegie Institute, Pittsburgh. 1950.

Spectra of the P Cygni Stars. C. S. Beals. Pub. Dominion Astrophysical Observatory, Victoria, B. C. Dept. Mines and Technical Surveys, Dominion Observatories, Ottawa. 1951.

State Economic Areas: A Description of the Procedure Used in Making a Functional Grouping of the Counties of the United States. Donald J. Bogue. GPO, Washington, D. C. 1951. $1.00.

University Research Potential: A Survey of the Resources for Scientific and Engineering Research in American Colleges and Universities. Engineering College Research Council, American Society for Engineering Education, Cambridge, Mass. June 1951.

The Value of Hormones in General Practice. W. N. Kemp. Burgess Pub., Minneapolis. 1949.

New Developments in Potassium and Cell Physiology: 1940-50[1]

C. W. Sheppard

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

POTASSIUM IS FOUND IN GREAT ABUNDANCE in plant and animal tissues, where it plays an important part in cellular function. Research on potassium has continued to be productive during the past decade, which began in 1940 with the Cold Spring Harbor Symposium on Permeability (*1*) and ended with the 1950 annual meeting of the Society of General Physiologists. At this meeting a special conference on electrolytes illustrated some of the gains made during this important period (*2*).

In 1940 the situation was summarized by Fenn (*3*). Potassium was known to be the most plentiful metallic cation in most animal cells and in many plant cells also. It was recognized to be essential for the growth and maintenance of man, the lower animals, and plants. Full recognition was given to the important role it plays in the physical biochemistry of proteins, in the physiology of excitable tissues, and in the electrochemistry of cells in general. Biologists were in agreement that to gain an increased understanding of the behavior of this element would be to contribute to many fields, including plant and animal physiology, cell physiology, endocrinology, and clinical medicine. The use of radioactive potassium as a tracer in biological studies had just begun, and pioneer experiments using this new tool were under way.

In discussing the contributions during the years that followed, a compromise must be made between the necessary conciseness required by the general reader and the detailed critical analysis desired by the specialist. Several recent reviews are recommended for those with more scholarly requirements. These include the discussions by Krogh (*4*) and Ussing (*5*), written from the point of view of general physiology, and the papers by Weller and Taylor (*6*) and Hoffman (*7*), which include some clinical considerations. The review by Overman (*8*) will meet some of the requirements of the endocrinologist, and the review by Steinbach (*9*) will answer many questions in muscle physiology. The more specific questions of permeability are discussed by Teorell (*10*), and neurophysiological considerations, by Lorente de Nó (*11*). For the nonspecialist, Fenn's recent popular review (*12*) is recommended.

Potassium and metabolism. In the earlier literature it was the fashion to regard potassium as a more or less passive metallic cation which responds in a biological system to the local concentration gradient and electrical field in its neighborhood. From this point of view its movement was thought to be controlled by the permeability or impermeability of membranes, and the element was believed to participate in biological phenomena primarily through its influence on the hydration of protoplasmic material. It is now becoming increasingly clear that potassium must be thought of in more complete terms as participating in enzymatic reactions, possibly as an essential element, and at the same time being accumulated into cells against ionic concentration gradients and electrical forces, deriving the necessary chemical free energy from metabolism. Many physiologists regard such processes as *active*, and the concept of active accumulation has been clearly presented in the review by Ussing (*5*).

It is now a familiar biochemical principle that various inorganic ions participate in the activation of particular enzyme systems. Potassium is required by the important enzyme enolphosphopyruvate–ADP transphosphorylase in muscle extracts (*13*). Without potassium the reaction has been shown to be irreversible (*14, 15*). A possibly related fact is that the element plays a significant role in the aerobic metabolism of brain (*16*), and increases the synthesis of glycogen from glucose in rabbit liver slices (*17*). It has been reported to depress the activity of saccharase, β-glycosidases, and catalase in molds and yeasts grown on potassium-containing media (*18*).

The symptoms of adrenal cortical insufficiency are the most familiar example of a relation between potassium and endocrine function. The relation between tissue potassium and the adrenal hormones has been reviewed by Overman (*8*). Recently a method has been described for determining small amounts of desoxycorticosterone with adrenalectomized rats, using the radioactive potassium isotope (*19*). The relation between potassium accumulation in cells and carbohydrate metabolism is still not clear. It has been known for many years that intravenous injections of epinephrine cause a rapid but transient increase in plasma potassium (*20*), which is particularly evident in arterial blood (*21*). In cats, injection by vein of those sugars, such as glucose, that are selectively absorbed in the intestine causes a transient rise in plasma potassium. This effect is removed by adrenalectomy (*22*). It was found in the early days of insulin therapy that injection of insulin causes a de-

[1] Work performed under Contract W-7405-Eng-26 for the Atomic Energy Commission. The author wishes to thank W. S. Wilde, Bernard L. Strehler, and L. J. Mullins for several references included in the bibliography.

pression in the plasma potassium level in animals (*23*) and in diabetic patients (*24*). This phenomenon continues to be useful in the clinical management of potassium disturbances.

The fact that many cells selectively accumulate potassium remains an unsolved problem in physiology, but the mere existence of a large potassium concentration gradient need not prove active accumulation of potassium directly. Our ideas concerning the selectivity of potassium accumulation in muscle have been strongly influenced by the Boyle-Conway theory, which has been reviewed by Conway (*25, 26*). According to this theory, potassium is accumulated in the muscle fiber passively as a result of a double Donnan effect. This effect arises from the electrical potential gradient set up by nonpenetrating colloidal anions inside the fiber and positive sodium ions that are excluded from the fiber in some way. Many writers now assume that sodium is kept out by a process of active extrusion. The active accumulation of potassium is thus thought to be indirect in this case.

The active accumulation of potassium either directly or indirectly has been observed in such widely different tissues as the chorion membrane of the hen's egg (*27*) and the isolated frog's heart (*28*). Recent experiments have shown that increased glucose utilization by rat diaphragm in the presence of added insulin is accompanied by an uptake of potassium after correction for leakage, perhaps caused in part by trauma of the excised tissue (*29*). In the process of active transport of potassium in brain tissue and in ox retina, glutamic acid is required in the medium, in addition to glucose (*30*). This has given rise to some interesting speculations concerning potassium absorption in the kidney (*31*). Active accumulation of potassium in yeast, although originally thought to be similar to that in animal tissue, is now known to be accompanied by a high production of acid and seems to proceed by the exchange of potassium for hydrogen ions (*32, 33*). It is a familiar fact that salt accumulation in plant cells is an active process intimately related to cellular metabolism. A recent discussion of the quantitative relation between salt accumulation and "salt respiration" includes a critical analysis of the Lundegårdh theory (*34*).

The maintenance of the resting potential of nerve by metabolic processes provides circumstantial evidence for active maintenance of ionic concentration gradients (*35*). Neurophysiologists have usually considered that sodium plays the active role in resting nerve. Recently Hodgkin and Katz (*36*) have provided strong evidence that, during passage of a nerve impulse, the reversal of polarization is due to the momentary shift of the active role from sodium to potassium. Frog sciatic nerve preparations lose more potassium in the absence of oxygen. Evidence was obtained that, when oxygen is present, accumulation may actually occur against a concentration gradient (*37*). It is becoming increasingly evident that potassium is lost from nerve during activity (*38*).

Isotope studies. The conclusive evidence that ionic concentration gradients in cells are not the result of membrane impermeability came from experiments with isotopically labeled ions. Pioneer studies with radioactive potassium have been reviewed by Hevesy (*39*), including the important early work by the University of Rochester investigators. The principal isotope used in studying the physiology of potassium has been K^{42}, since it has the advantage of a highly penetrating β-ray that minimizes self-absorption difficulties in counting. Although the associated γ-ray is weak, the β-ray activities could in principle be determined in a thick-walled ionization chamber by the Bremsstrahlung method of Tompkins *et al.* (*40*). K^{42} has been employed successfully in macroscopic radioautography (*41*). The short half-life (12.4 hours) precludes its use in long-term studies and complicates its use at a distance from the source of production.

Recently it has been shown that, under certain conditions, the reactor-activated isotope can contain detectable quantities of long-lived contaminants in addition to K^{40}. These include Rb^{86} caused by the presence of small amounts of the parent element in the irradiated material (*42*). Such contaminants usually appear when the material has been exposed to slow neutrons for periods of a week or more, followed by an extended period of decay. The use of reactor-produced K^{42} (*43*) greatly lessens the problem of Na^{24} contamination; nevertheless, the formation of the latter isotope is still favored by a factor of 20, and the parent material must be practically sodium-free, unless a radiochemical purification is to be used.

Where the short-lived isotope is unacceptable, it becomes necessary to resort to the naturally occurring radioactive isotope K^{40} obtained by isotope separation methods. In this case the usual radiochemical precautions in the use of a long-lived istotope become necessary. According to Mullins and Zerahn (*44*), analyses of the normal K^{40} content of various animal, vegetable, and mineral sources showed no variation within 0.5 per cent.

Since the effectiveness of isotope experiments is greatly increased by simultaneous chemical determinations, recent improvements in the methods of alkali metal analysis are of interest. Flame spectroscopy is not a new technique, having been used in biology by Lundegårdh more than twenty years ago. The fact that the flame photometer has only recently been perfected as a routine commercial instrument (*45*) illustrates a continuing lack of direct contact between some fields of physical instrumentation and biochemistry. With this instrument, potassium determinations of acceptable accuracy are completed in hours, compared with days for the chemical methods.

An increasing number of papers describing tracer experiments with K^{42} are appearing in the literature. On the assumption that earlier work has been discussed adequately in existing reviews, only recent reports will be considered. These cover a variety of biological experiments. In the field of marine biology, echinoderm eggs take up a strikingly larger amount of radioactive potassium from artificial sea water

when they are fertilized than do the unfertilized controls (*46*). Studies of chicken embryo muscle by the tissue culture method show that the potassium does not move into and out of the cells as though they were a single, uniformly mixed compartment. The results indicate either that potassium is present in more than one chemical state or, more probably, that there are structural inhomogeneities in the cells (*47*). Cultures of *Escherichia coli* show a labile potassium fraction that exchanges completely with the potassium of the suspension medium in less than five minutes, and a tightly bound fraction that increases as metabolism progresses (*48*). Sections of squid nerve, when suspended in artificial sea water, rapidly exchange about 10 per cent of their potassium in one to two hours, the remainder seemingly being slowly exchangeable, if at all (*49*). One limitation of experiments with material that is structurally intact, or nearly so, is that, if a bound fraction exists, in principle it cannot be identified as being chemically rather than physically bound. The decision requires additional information.

The inhomogeneities that occur in cellular systems do not always present an insurmountable obstacle to quantitative experimentation. Harris and Burn (*50*) have considered experiments on the penetration of labeled sodium or potassium into excised muscle suspended in solutions containing Na^{24} or K^{42}, where one must consider both the diffusion of ions in the extracellular space and the rate of crossing cell boundaries. They present an approximate mathematical solution to the problem based on the now familiar analogy between the movement of radioactive tracers in a closed steady state system and the classical problem of the flow of heat. This analogy has been recently discussed by Sheppard and Householder (*51*).

The permeability of mammalian erythrocytes to cations was investigated soon after the first successful large-scale cyclotron production of isotopes. This problem has continued to receive attention, the most clear-cut results having been obtained for potassium in human erythrocytes. Here independent investigations in two different laboratories have achieved remarkably good agreement (*43, 52*). When freshly drawn heparinized human blood is equilibrated at 38° C under an atmosphere containing 5 per cent CO_2 in the presence of added sugar, the entire cellular potassium exchanges at a uniform rate, the specific activity changes of cells and plasma proceeding as though the erythrocyte potassium were in a single pool. The exchange rate under normal conditions is 1.6–1.8 per cent of the cellular potassium per hour. Between 44° C and 15° C the logarithm of the exchange rate is proportional to the reciprocal of the absolute temperature with a Q_{10} of 2.35. The kinetics are unaltered by oxygenation or reduction of the hemoglobin or exposure to 1,200 r of γ-rays. Below 15° C the familiar net leakage of potassium from the cells ensues; above 44° C the system rapidly deteriorates.

The observation of Raker *et al.* (*52*) that the rate of exchange is essentially unaltered by increasing the plasma potassium concentration has been confirmed more recently (*53*). Danish investigators (*54*) report that increasing the plasma potassium roughly threefold produces about an 8:5 increase in rate. Whatever the explanation of the disagreement, the relation between concentration and exchange rate is not that of a passive diffusion of potassium. In the potassium-depleted cells of hypopotassemia the uptake of the element is due to an increased influx rather than to an arrested outgo (*54*).

Although potassium does exchange in the erythrocytes of other species, the quantitative description of the process is less satisfactory. Human cells show a stability *in vitro* that is often lacking in other red cells, particularly those of the dog. Often it is not possible to continue an experiment long enough to test the completion of exchange. As in the chicken embryo muscle experiments, inhomogeneities in the exchange rates are often found. In canine blood one such cause of multiplicity is the exchange in the cells of the buffy coat (*53*). Isotope experiments on red cells *in vivo* are complicated by the exchange of potassium in other body tissues, although semiquantitative kinetic studies are possible.

Isotope experiments *in vivo* have the advantage that they produce minimal disturbance of the tissue under investigation. When potassium containing a radioactive label is injected into the circulation of an animal, it will appear in various tissues at different rates. Early studies in the rat (*55*) showed a wide variation in the appearance rate, the liver and kidney being among the most rapid, muscle tissue being intermediate, and erythrocytes and brain being slow. Such investigations are now being pursued more thoroughly in the rabbit (*56*). The most striking phenomenon is the extremely precipitous fall in the circulating level of intravenously injected radioactive potassium. In order to observe the decline quantitatively, samples must be obtained seconds apart following injection. Since these times are small compared to the circulation time it must be accepted that the rate of removal of the isotope by exchange of potassium with that in the tissues is now limited by the circulation rate, which controls the speed of delivery of the isotope to the site of removal. It has been repeatedly observed that the specific activity of the liver exceeds that of the plasma for a considerable period shortly after injection, showing that in this organ the mixing of injected potassium with that of the organ does not proceed by simple passive diffusion. The penetration of potassium into normal and atrophied rabbit muscle has been observed by Fischer *et al.* (*57*), who noted a tendency for the muscle specific activity to overshoot that of the plasma.

It is well to inject a word of caution concerning the interpretation of experiments on the kinetics of disappearance of injected substances from the circulation. Observations of the circulating radioactivity alone are, in principle, not sufficient uniquely to determine exchange rates between the circulation and other body compartments.

Experiments with isotopes, such as K^{42}, which are rapidly removed from the circulation when injected, have recently called attention to the mechanics of the circulatory mixing process. An ingenious method has been developed by Morel (*58*) whereby the activity of the blood may be continuously recorded following injection. Working with Na^{24}, it was shown that the first twenty seconds of the disappearance curve of this isotope are complicated by the presence of a wave of activity during the process of mixing. These considerations apply to potassium experiments as well.

Potassium accumulation in erythrocytes. The potassium metabolism of human erythrocytes has been of considerable practical interest in connection with studies of blood preservation both here and abroad (*59–62*). The behavior of potassium is of considerable fundamental interest, however, since it is not unlikely that processes that occur in a relatively simple cellular system, such as the erythrocyte, may resemble processes in other tissues. These processes of cation control are intimately related to the maintenance of osmotic stability of cells and, in excitable tissues, to the production of bioelectric phenomena. For the period prior to 1942 most of the literature on the transport of potassium in cellular systems, including erythrocytes, is reviewed in the monograph by Davson and Danielli (*63*), where a number of now familiar principles are discussed and documented. One point that may be recalled is the large species variation in the selective accumulation of potassium by mammalian red cells, ranging from practically no accumulation in the cells of the dog to a nearly twenty-fold concentration ratio between cells and plasma in the case of human blood. Familiar also is the tendency for high-potassium cells to lose potassium when suspended in nonelectrolyte solutions or in media containing a wide variety of mildly injurious substances, such as glycolytic inhibitors, lytic agents, heavy metals, and rose bengal activated by light.

It has recently been shown by Ponder (*64*) that in some of these disturbances the progressive potassium loss is accompanied by an almost equivalent penetration of sodium. Overman (*65*) has reported the exchange of sodium for potassium in nonparasitized erythrocytes of the malarious monkey. Reciprocal potassium leakage and sodium penetration have also been noted in human red cells when exposed to relatively high doses of x-rays (*66*). The exchange is not confined to red cells alone. Reciprocal exchange of sodium for potassium has also been observed in anoxic nerve (*67*), and good evidence exists that it also occurs in rabbit leucocytes under varying sugar concentrations (*68*). Recent studies with liver slices suggest that the exchange occurs in hepatic tissue (*69*). It is thus apparent that a specific failure of potassium selectivity must be included among the injurious effects that can occur in erythrocytes and other tissues.

Experiments on the exchange of potassium for sodium in red cells deal primarily with the movement of sodium and potassium from regions of high to regions of low concentration. The reverse process requires a source of chemical free energy doubtless derived from metabolic processes. It was first reported by Danowski in freshly drawn defibrinated human blood (*70*) and by Harris in human cells which were depleted of their potassium by low temperature storage (*71*). These studies both showed that, at 38° C in the presence of sugar, potassium enters the cells, the current being reversed as the sugar concentration approaches zero. Addition of sugar prolongs the period before potassium leakage sets in. Upon addition of fluoride an irreversible potassium leakage from the cells occurs. Danowski showed that the normal movement of potassium was not accompanied by large water transfers, and Harris showed that this was due to the reciprocal movement of sodium. More recently the phenomenon was re-examined by Maizels (*72*), who demonstrated that the accumulation process has a pronounced pH optimum at about 7.4. The view was favored that the process was essentially one of active sodium exclusion. That sodium transport in red cells is indeed related in some way to an active process is suggested by the pH and temperature sensitivity observed in the studies of Davson (*73*) and Davson and Reiner (*74*) on the loss of sodium from cat erythrocytes suspended in isotonic NaCl; nevertheless, sodium exclusion alone cannot be accepted in the erythrocyte without modification. The recent investigation by Ponder (*75*) demonstrates that accumulation of potassium occurs almost as well when sodium in the external environment is replaced by lithium or cesium. Ponder found that the optimal glucose concentration lies in the region 50–200 mg per cent and that the Q_{10} of the process is 2.4. Maizels searched for a relationship between potassium accumulation and the breakdown of organic phosphorous compounds without obtaining clear-cut results. It is of interest to recall that in the earlier literature Kerr (*76*) noted a correlation between the intracellular potassium content in the erythrocytes of several species and the organic acid soluble phosphorus. An interesting aspect of these experiments is that all the potassium does not exchange by the same amount. This has been discussed recently by Ponder, who stresses the effect of nonuniformity among the cell population (*77*).

Perhaps the most interesting recent contribution to the problem of potassium accumulation in erythrocytes has been the discovery by Greig and Holland (*78*) that the specific cholinesterase inhibitor physostigmine disturbs the accumulation process. In experiments by the isotope technique Taylor and Weller claim that cholinesterase inhibitors depress the rate of penetration of potassium, whereas inhibitors known to affect choline acetylase increase the rate of loss from cells to plasma (*79*). Washed cells which are provided with acetyl choline remain intact *in vitro* for longer periods of time than those to which substrate is denied (*80*). Recent observations by Russian workers also indicate a correlation between the activity of cholinesterase in erythrocytes and their permeability (*81*). The assignment of a role for the

cholinesterase associated with the red cell envelope (*82*) thus establishes an unexpected similarity between ionic effects in erythrocytes and in excitable tissues.

Mechanism of selective accumulation. Following presentation of the conclusive evidence that potassium is accumulated directly or indirectly by active processes in most, if not all, biological systems, it is pertinent to inquire into the mechanism by which the element is concentrated in cells against the concentration gradient. One suggestion that is occasionally offered is that potassium in the cell exists in some chemically bound form. Indeed, in the case of *E. coli* the evidence favors some potassium binding. In animal tissues the concept of binding of alkali cations remains in an equivocal state. Nondialyzable potassium fractions have been reported in extracts of rat brain and muscle (*83*). That these observations may be due to an artifact is suggested by the lack of a comparable effect in frog muscle homogenates, where the sedimentable fraction contains, if anything, a slight excess of sodium (*84*). Little is known about the tendency of alkali metals to form undissociated chemical compounds; however, Christensen and Hastings describe such a compound between sodium or potassium and cephalin (*85*).

The concept of bound potassium fails to give a satisfactory explanation of accumulation in erythrocytes. In human cells the isotope results show that all the intracellular potassium exchanges as though it were in a single pool. It would thus be necessary to postulate that all the intracellular cation (mostly potassium) was bound but slowly exchangeable. Although the erythrocyte is not a perfect osmometer, the binding of all the intracellular cation would certainly be reflected in its osmotic behavior. It would also seem to be no accident that the intracellular concentration of total cations in species with widely different intracellular potassium is quite constant and not far different from that of the plasma, where the alkali cations are essentially entirely free (*86*).

It may be argued that the forces between cations and the intracellular material are as yet incompletely understood. Such forces might be selective for potassium, and such a hypothetical effect coupled with an internal resistance to water transfer might fit the experimental facts and yet permit potassium accumulation in erythrocytes by a binding process. The osmotic deficit would be compensated for by the binding of water, so that the cation is bound in an isosmotic solution. Certainly the space occupied by hemoglobin in the cell is great, and the ions are at all times close to protein molecules. The argument thus rests on how radically this proximity alters the thermodynamic activity of the ions and of the water molecules. Some evidence on this question was recently obtained by Stratmann and Wright (*87*), who found that hemoglobin solutions, when dialyzed against semipermeable membranes, could accumulate potassium. The less than threefold increases in concentration they obtained were much less than those occurring in high potassium erythrocytes. A similar conclusion can be drawn for the accumulation of potassium by myosin (*88*). Although such accumulations are not impressive, their results cannot be lightly discarded without further investigation.

The Donnan theory provides a ready explanation for the relatively small static concentration gradient which classical thermodynamics predicts for ions when a semipermeable membrane constrains a solution of impermeable electrically charged molecules. It has been shown that such a gradient will also arise as a result of active transport of ions (*89, 90*). The question whether this mechanism alone could account for selective potassium accumulation was discussed by Spiegelman and Reiner (*91*), who concluded that the unmodified classical theory was unable to account for potassium selectivity. Denying that differences in the mobility of sodium and potassium ions can account for it, they concluded that a specific chemical force of some type acting on sodium or potassium, or both, was required to explain the observed facts. Such a force has been postulated for sodium alone in muscle by the Boyle-Conway theory (*25, 26*). The thesis that potassium is passive rests on such experiments as those of Wilde, who has shown that, when the plasma potassium is elevated in nephrectomized rats by feeding potassium, there is an increase in the muscle fiber potassium, presumably as a passive result of the concentration increase (*92*). That sodium is actively excluded (*5*) is based on the ability of animals with elevated muscle sodium produced by a low potassium diet to extrude this sodium when the dietary potassium is increased (*93*).

In the erythrocyte the modified Donnan theory presents a difficulty, if it be postulated that potassium is accumulated passively as a consequence of active sodium exclusion. If potassium is regarded as a passive ion, then according to the classical picture it will be distributed across the cell membrane in conformance with the Donnan ratio. This would require a variable ratio for the red cells of different species ranging from nearly one for canine cells to nearly twenty for the cells of man. Correspondingly, the chloride ratios and intracellular pH would vary between these wide limits. Although ratios of two are observed, a tenfold greater ratio will not fit the experimental facts. The same argument holds for passive control of sodium as well.

One promising explanation for the selectivity which has received considerable attention assumes that a process occurs at the cell interface in which the rate of transport of the cation inward is made to exceed the rate outward. A particular version of this point of view has been maintained for some time by Osterhout (*94*) and recently summarized. He postulates that potassium is carried into the cell by some carrier molecule, from which it dissociates on the intracellular side of the interface. The Lundegårdh theory for ion transport in plant cells (*34*) has been cited freely during the past decade. This postulated the existence of a potential difference across the plant wall as a

result of a redox reaction involving cytochrome. A more general theory that retains cytochrome as the redox system has recently been advanced by Conway (*95*) and Conway, Brady, and Carton, for the exchange of potassium for hydrogen ions in yeast and other acid- or alkali-forming tissues (*96*).

Although many ingenious attempts have been made during the past decade, the achievements in the potassium field do not include the establishment of a satisfactory theory of the maintenance of ionic concentration gradients in cells. However, some of the basic considerations that must be included in such a theory have been discussed. Definite progress has been made in recognizing the existence of active accumulation processes, in the realization of their widespread nature, and in their close connection with cell metabolism. The use of isotopically labeled substances has demonstrated in an unequivocal fashion the movement of cations into and out of cells which were postulated by earlier workers to be cation-impermeable. The first flush of ambition among isotope workers has been replaced with a more mature caution, and there is increased consciousness of what is easy and what is difficult in this field. Cell physiologists have obtained a larger body of information relating the physical aspects of cation movement to cellular biochemistry. The biochemist alone has been unable to explain all the factors controlling ionic movement. Nevertheless, he can say in certain instances how the necessary energy for the process is mobilized in the cell, and he can cite one possible use for the element after it is accumulated. It is left as a task for the future to establish how the cation interacts at the cell interface, how the energy is expended in the movement of the ion into the cell, and how the ion later leaves the cell in exchange for another.

References

1. *Cold Spring Harbor Symposia Quant. Biol.*, **8**, (1940).
2. Papers presented at meeting Soc. Gen. Physiologists, *Biol. Bull.*, **99**, 308 (1950).
3. Fenn, W. O. *Physiol. Revs.*, **20**, 377 (1940).
4. Krogh, A. *Proc. Roy. Soc. (London)*, **B133**, 140 (1946).
5. Ussing, H. H. *Physiol. Revs.*, **29**, 127 (1949).
6. Weller, J. M., and Taylor, I. M. *Ann. Internal Med.*, **33**, 607 (1950).
7. Hoffman, W. S. *J. Am. Med. Assoc.*, **144**, 1157 (1950).
8. Overman, R. R. *Physiol. Revs.* (in press).
9. Steinbach, H. B. *Ann. N. Y. Acad. Sci.*, **47**, 849 (1947).
10. Teorell, T. *Ann. Rev. Physiol.*, **11**, 545 (1949).
11. Lorente de No, R. *Rockefeller Inst. Med. Research Studies*, **131**, Suppl. 496, **132**, Suppl. 548 (1947).
12. Fenn, W. O. *Sci. American*, **181**, (2), 16 (1949).
13. Boyer, P. D., Lardy, H. A., and Phillips, P. H. *J. Biol. Chem.*, **146**, 673 (1942).
14. Lardy, H., and Ziegler, J. *Ibid.*, **159**, 343 (1945).
15. Meyerhof, O., and Oesper, P. *Ibid.*, **179**, 1371 (1949).
16. Lipsett, M. N., and Crescitelli, F. *Arch. Biochem.*, **28**, 329 (1950).
17. Buchanan, J. M., Hastings, A. B., and Nesbett, F. B. *J Biol. Chem.*, **180**, 435 (1949).
18. Hofmann, E., and Scheck, H. *Biochem. Z.*, **321**, 98 (1950).
19. Dorfman, R. I. *Proc. Soc. Exptl. Biol. Med.*, **70**, 732 (1949).
20. D'Silva, J. L. *J. Physiol. (London)*, **82**, 393 (1934).
21. Brewer, G., Larson, P. S., and Schroeder, A. R. *Am. J. Physiol.*, **126**, 708 (1939).
22. Verzar, F., and Somogyi, J. C. *Nature*, **144**, 1014 (1939).
23. Briggs, A. P., *et al. J. Biol. Chem.*, **58**, 721 (1923).
24. Harrop, G. A., and Benedict, E. M. *Ibid.*, **59**, 683 (1924).
25. Conway, E. J. *Irish J. Med. Sci.*, **6**, 593 (1947).
26. ———. *Biol. Revs. Cambridge Phil. Soc.*, **20**, 56 (1945).
27. Krogh, A. *Acta Physiol. Scand.*, **6**, 203 (1943).
28. Krogh, A., Lindberg, A. L., and Schmidt-Nielsen, B. *Ibid.*, **7**, 221 (1944).
29. Willebrands, A. F., *et al. Science*, **112**, 277 (1950).
30. Terner, C., Eggleston, L. V., and Krebs, H. A. *Biochem. J.*, **47**, 139 (1950).
31. Binkley, F. In S. E. Bradley (Ed.), *Renal Function*. New York: Josiah Macy, Jr. Foundation (1949).
32. Conway, E. J., and Brady, T. G. *Biochem. J.*, **47**, 360 (1950).
33. Rothstein, A., and Enns, L. H. *J. Cellular Comp. Physiol.*, **28**, 231 (1946).
34. Robertson, R. N., and Wilkins, M. J. *Australian J. Sci. Research, Ser. B, Biol. Sciences*, **1**, 17 (1948).
35. Blair, H. A. *Ann. Rev. Physiol.*, **12**, 399 (1950).
36. Hodgkin, A. L., and Katz, B. *J. Physiol. (London)*, **108**, 37 (1949).
37. Fenn, W. O., and Gerschman, R. *J. Gen. Physiol.*, **33**, 195 (1950).
38. Hodgkin, A. L., and Huxley, A. F. *J. Physiol. (London)*, **106**, 341 (1947).
39. Hevesy, G. *Radioactive Indicators, Their Application in Biochemistry, Animal Physiology, and Pathology*. New York: Interscience (1948).
40. Tompkins, P. C., Wish, L., and Burnett, W. T. *Anal. Chemi.*, **22**, 672 (1950).
41. Colfer, H. F., and Essex, H. E. *Proc. Soc. Exptl. Biol. Med.*, **63**, 243 (1946).
42. Corsa, L., *et al. J. Clin. Invest.*, **29**, 1280 (1950).
43. Sheppard, C. W., and Martin, W. R. *J. Gen. Physiol.*, **33**, 703 (1950).
44. Mullins, L. J., and Zerahn, K. *J. Biol. Chem.*, **174**, 107 (1948).
45. Berry, J. W., Chappell, D. G., and Barnes, R. B. *Ind. Eng. Chem., Anal. Ed.*, **18**, 19 (1946).
46. Chambers, E. L., *et al. Biol. Bull.*, **95**, 252 (1948).
47. Wesson, L. G., Cohn, W. E., and Brues, A. M. *J. Gen. Physiol.*, **32**, 511 (1949).
48. Cowie, D. B., Roberts, R. B., and Roberts, I. Z. *J. Cellular Comp. Physiol.*, **34**, 243 (1949).
49. Rothenberg, M. A. *Biochim. et Biophys. Acta*, **4**, 96 (1950).
50. Harris, E. J., and Burn, G. P. *Trans. Faraday Soc.*, **45**, 508 (1949).
51. Sheppard, C. W., and Householder, A. S. *J. Applied Phys.* (in press).
52. Raker, J. W., *et al. J. Gen. Physiol.*, **33**, 691 (1950).
53. Sheppard, C. W., Martin, W. R., and Beyl, G. E. *Ibid.*, **34**, 411 (1951).
54. Davidsen, H. G., and Kjerulf-Jensen, K. *Proc. Soc. Exptl. Biol. Med.*, **74**, 477 (1950).
55. Noonan, T. R., Fenn, W. O., and Haege, L. *Am. J. Physiol.*, **132**, 474 (1941).
56. Walker, W. G., and Wilde, W. S. *Ibid.*, **163**, 759 (1950).
57. Fischer, E., *et al. Arch. Phys. Med.*, **31**, 429 (1950).
58. Morel, F. F. *Helv. Physiol. et Pharmacol. Acta*, **8**, 52 (1950).
59. DeGowin, E. L., Harris, J. E., and Plass, E. D. *J. Am. Med. Assoc.*, **114**, 855 (1940).
60. Poznanskaya, N. B. *Byull. Eksp. Biol. Med.*, **17**, 22 (1944).
61. Poli, E., and Corio, G. *Boll. soc. ital. biol. sper.*, **21**, 31 (1946).
62. Kiersz, J. *Arch. intern. pharmacodynamie*, **74**, (215), 212 (1947).
63. Davson, H., and Danielli, J. F. *The Permeability of Natural Membranes*. New York: Cambridge Univ. Press (1943).
64. Ponder, E. *J. Gen. Physiol.*, **30**, 379 (1947).
65. Overman, R. R., and Davis, A. K. *Am. J. Physiol.*, **152**, 113 (1948).
66. Sheppard, C. W., and Beyl, G. E. *J. Gen. Physiol.*, **34**, 691 (1951).
67. Shanes, A. M. *Biol. Bull.*, **99**, 309 (1950).
68. Manery, J. F., *et al. Ibid.*, 312.
69. Flink, E. B., Hastings, A. B., and Lowry, J. K. *Am. J. Physiol.*, **163**, 598 (1950).
70. Danowski, T. S. *J. Biol. Chem.*, **139**, 693 (1941).
71. Harris, J. E. *Ibid.*, **141**, 579 (1941).
72. Maizels, M. *J. Physiol. (London)*, **108**, 247 (1949).
73. Davson, H. *Cold Spring Harbor Symposia Quant. Biol.*, **8**, 255 (1940).

74. Davson, H., and Reiner, J. M. *J. Cellular Comp. Physiol.*, **20**, 325 (1942).
75. Ponder, E. *J. Gen. Physiol.*, **33**, 745 (1950).
76. Kerr, S. E. *J. Biol. Chem.*, **117**, 227 (1937).
77. Ponder, E. *J. Gen. Physiol.*, **34**, 359 (1951).
78. Greig, M. E., and Holland, W. C. *Arch. Biochem.*, **23**, 370 (1949).
79. Taylor, I. M., and Weller, J. M. *Biol. Bull.*, **99**, 311 (1950).
80. Greig, M. E. *Ibid.*
81. Koshtoyants, K. S., and Bulatova, N. N. *Doklady Acad. Nauk S.S.S.R.*, **71**, 199 (1950).
82. Paleus, S. *Arch. Biochem.*, **12**, 153 (1947).
83. Stone, D., and Shapiro, S. *Am. J. Physiol.*, **155**, 141 (1948).
84. Steinbach, H. B. *Ibid.*, **163**, 236 (1950).
85. Christensen, H. N., and Hastings, A. B. *J. Biol. Chem.*, **136**, 387 (1940).
86. Folk, B. P., Zierler, K. L., and Lilienthal, J. L. *Am. J. Physiol.*, **153**, 381 (1948).
87. Stratmann, C. J., and Wright, R. D. *Australian J. Exptl. Biol. Med. Sci.*, **26**, 493 (1948).
88. Mullins, L. J. *Federation Proc.*, **1**, (1), Part II, 61 (1942).
89. Wilbrandt, W. *Arch. ges. Physiol. (Pflügers)*, **246**, 274 (1942).
90. Teorell, T. *Proc. Natl. Acad. Sci. U. S.*, **21**, 152 (1935).
91. Spiegelman, S., and Reiner, J. M. *Growth*, **6**, 367 (1942).
92. Wilde, W. S. *Am. J. Physiol.*, **143**, 666 (1945).
93. Conway, E. J., and Hingerty, D. *Biochem. J.*, **42**, 372 (1948).
94. Osterhout, W. J. V. *Biol. Bull.*, **99**, 308 (1950).
95. Conway, E. J. *Science*, **113**, 270 (1951).
96. Conway, E. J., Brady, T. G., and Carton, E. *Biochem. J.*, **47**, 369 (1950).

Technical Papers

Natural Black Uranium Powder

Paul F. Kerr[1]

Department of Geology,
Columbia University, New York

A soft, black, uranium-bearing powder has been found in several localities during the exploration of uranium deposits in the western United States. The nature of this powder, its significance from the standpoint of origin, and its distribution constitute matters of scientific interest, as well as of practical importance. Among the localities in which the powder has been particularly noted, specimens were collected for study from Marysvale and White Canyon, Utah, and the Caribou and Bellvue-Rochester mines in Colorado. Material of similar appearance has also been noted in a collection of specimens from the Belgian Congo.

The soft pulverulent character of the material and the color suggest that it might be a form of the organic uranian mineral thucholite, recognized by Ellsworth (*1*) as a black uranian hydrocarbon in rocks of the Canadian shield. However, analyses for carbon (Table 1) made by the New Brunswick laboratory of the AEC yield amounts so low that thucholite could hardly be present in any significant amount.

The material in question is not thucholite, and laboratory studies indicate that it is largely uraninite. The various pulverulent materials collected yield substantial amounts of uranium on the basis of x-ray fluorescence analysis, with the use of a technique similar to that described by Birks and Brooks (*2*). Moreover, diffraction patterns yield interplanar spacings that establish the prevailing crystalline uranian constituent of the powder as uraninite. The material is commonly

[1] In the conduct of these studies the writer has had the benefit of cooperation by C. J. Rodden, chief, Microchemical Branch, New Brunswick Laboratory, AEC, and Harold Wright, who is studying the mineral relationships at the Caribou mine.

TABLE 1

Carbon Content of Sooty Uraninite Samples

	A (%)	B (%)
Bellvue-Rochester mine, Colo.	0.12	
White Canyon, Utah	0.15	0.09
Marysvale, Utah	0.18	0.13

mixed with fine pyrite, and at some localities other metallic sulfides are associated.

It has been observed that certain samples of uraninite from the Shinkolobwe mine in the Belgian Congo are also sooty, and one specimen with slickensides even appears graphitic. On analysis these samples are likewise shown to be noncarbon-bearing. Such specimens yield x-ray diffraction patterns corresponding to uraninite but with low lattice constants, in contrast to the lattice constants of hard cubic crystals of uraninite from the same locality (Table 2).

A number of observations indicate that the sooty uraninite may be a later form high in UO_3 that has originated at the expense of earlier hard uraninite, high in UO_2. Ellsworth (*3*) studied the successive zones in a large, progressively altered uraninite crystal from Villeneuve, Quebec, and pointed out that UO_2 and total U decreased from the center outward but that, at the same time, UO_3 notably increased until accountable for the entire uranium content at the most highly altered surface. Kidd and Hayecock (*4*), in their study of the ores of Great Bear Lake, noted a later type of uraninite, less lustrous and softer than original hard uraninite, and formed at the expense of the earlier mineral. In the earlier uraninite the ratio $UO_2 : UO_3$ was 10 : 2.2, but in the later uraninite the ratio was 1 : 10. Analyses of both sooty and graphitic

TABLE 2

Lattice Constants, Shinkolobwe Uraninite

Well-formed cubic crystal	5.453 A.U.
Graphitic type	5.438
Sooty type	5.411

types of pulverulent uraninite from Shinkolobwe suggest a high UO_3 content for this material. At Marysvale and Caribou hard, brittle uraninite has been found in association with the sooty material, the latter coating and penetrating the solid brittle ore.

Although the sooty mineral is later, it is found at such depths that it apparently does not represent a typical surface-weathering product. At White Canyon it is found 50–100 ft underground from the tunnel portal but not directly at the surface. At Marysvale, it is found a corresponding depth below the surface and, in addition, in lower mine workings in the Prospector mine. At Caribou the sooty mineral occurs at the 1,040-ft level and below. At Bellvue-Rochester it lies far below the surface.

The environment in which the sooty mineral is found is more or less porous and permeable to solutions. At the two Colorado localities and at Marysvale, it occurs in an envelope of fractured clay, having a thickness of several feet on either side of the uranium-bearing core where the enclosing wall rock has been altered to clay and associated porous minerals. At White Canyon the wall rock is a more or less horizontal stratum of porous sandstone about 10 ft thick, with shale above and below. The porous character of the environment in which the black powder is found, even including the associated host rock on the Shinkolobwe specimens, indicates an enclosing zone permeable to solutions. Observations at the surface itself and a few feet below the outcrop are inconsistent with the assumption that the sooty mineral is the result of simple weathering, since the pulverulent material, if formed in such a way, should persist to the surface. In the outcrop zone at Marysvale and White Canyon, yellow or green oxidized uranium minerals, typical of surface weathering, occur in place of the sooty material.

Associated metallic sulfides of copper at White Canyon, and zinc and lead as well at Caribou, point to the likelihood that the originating temperatures were higher than would prevail for normal ground water. Indicators belonging to the temperature scale of the geologic thermometer have been observed in the form of vein fluorite, chalcopyrite, and sphalerite, but the temperatures of formation are indefinite.

While studies are still in progress and further publication is in preparation, it seems reasonable to conclude that solutions and complete submersion, rather than simple weathering and surface oxidation, must be assumed responsible for the sooty uraninite. Since the mineral is prevailingly uraninite and occurs in a hydrothermal environment, it also seems reasonable to suggest that the solutions from which precipitation occurred were heated.

References

1. Ellsworth, H. V. *Am. Mineral.*, **13**, 419 (1928).
2. Birks, L. S., and Brooks, E. J. *Anal. Chem.*, **22**, 1017 (1950).
3. Ellsworth, H. V. *Rare-Element Minerals of Canada.* Canada Dept. Mines, Geol. Survey, Econ. Geol. Ser., 242 (1932).
4. Kidd, D. F., and Haycock, M. H. *Bull. Geol. Soc. Am.*, **46**, 901 (1935).

Plant Growth-regulating Activity in Certain Aryloxyalkylcarboxylic Acids

D. J. Osborne and R. L. Wain

Wye College, University of London, Ashford, Kent, England

In our first report on substitution into the side chain of certain aryloxyacetic acids (*1*), it was suggested that the presence of a hydrogen atom on the carbon adjacent to the carboxyl group may be necessary for certain types of plant growth-regulating activity. Further compounds have now been synthesized, the assessment of biological activity has been extended by the inclusion of other tests, and additional evidence obtained to support our original suggestion. The following classes have been investigated, in which the aryl groups are phenyl, 2-chlorophenyl, 4-chlorophenyl, 2 : 4-dichlorophenyl, 2-methyl-4-chlorophenyl, 2 : 4 : 5-trichlorophenyl, 1-naphthyl, and 2-naphthyl:

$ArOCH_2COOH$ (I) aryloxyacetic acid	$ArOCH(CH_3)COOH$ (II) α-(aryloxy)-propionic acid
$ArOCH(C_2H_5)COOH$ (III) α-(aryloxy)-*n*-butyric acid	$ArOC(CH_3)_2COOH$ (IV) α-(aryloxy)-*iso*butyric acid

It was previously shown (*1*) that four aryloxy*iso*butyric acids failed to induce responses in the tomato leaf epinasty test (*2*). These findings have been confirmed, all the eight *iso*butyric acids listed above being inactive in this test, whereas with the exception of phenoxyacetic acid, all the acetic, propionic, and *n*-butyric acids (I, II, and III) were active.

Other methods we have employed for assessing growth-regulating activity have included the *Avena* curvature (*3*), *Avena* cylinder (*4*), tomato parthenocarpy (*5*), tomato leaf rooting (*6*), and Went pea curvature (*7*) tests. In addition, the capacity to induce morphological changes in the growth of tomato plants has been studied (*8*).

In general, it was found that compounds that possess at least one hydrogen attached to the α-carbon of the side chain (I, II, and III) give a positive response in the tomato leaf epinasty, *Avena* curvature, and *Avena* cylinder tests, all of which depend upon cell elongation. Compounds in which such hydrogen atoms had been substituted by methyl groups (IV) were found to be uniformly inactive in these tests at the concentrations employed; indeed, certain of the compounds appeared to inhibit the normal growth of cells.

In tests involving cell division—e.g., tomato parthenocarpy, leaf rooting, and production of morphological effects—most of the *iso*butyric acids (IV) were inactive. However, 2 : 4-dichloro-, 2-methyl-4-chloro-, and 2 : 4 : 5-trichlorophenoxy*iso*butyric acids showed

some, though in all cases a lower, activity than the corresponding acetic, propionic, and *n*-butyric acids.

Within the range of acids tested, all acetic, propionic, and *n*-butyric derivatives (I, II, III) with the exception of phenoxyacetic acid, induced positive curvatures in the pea test. Some activity was also shown by certain *iso*butyric acids (IV) in this test, though phenoxy-, 2-chloro-, and 4-chlorophenoxy*iso*butyric acids were inactive. The pea test, however, cannot be classified as assessing simple cell extension or cell division (*8*). A more detailed study of all these compounds in the pea test is now the subject of a separate investigation.

Our results indicate that, in general, the α-(aryloxy)-*iso*butyric acid structure is not associated with high growth-regulating activity. In particular, such compounds do not stimulate cell extension in the test methods we have employed.

Further details of this work will be published elsewhere.

References

1. Osborne, D. J., and Wain, R. L. *Proc. IInd Intern. Cong. Crop Protect., London, 1949.* (In press.)
2. Zimmerman, P. W., and Hitchcock, A. E. *Contribs. Boyce Thompson Inst.*, **12**, 321 (1942).
3. Went, F. W. *Rec. trav. botan. néerland.*, **25**, 1 (1928).
4. Bonner, J. *J. Gen. Physiol.*, **17**, 63 (1933).
5. Zimmerman, P. W., and Hitchcock, A. E. *Proc. Am. Soc. Hort. Sci.*, **45**, 353 (1944).
6. Hitchcock, A. E., and Zimmerman, P. W. *Contribs. Boyce Thompson Inst.*, **9**, 463 (1938).
7. Went, F. W. *Proc. Acad. Sci. Amsterdam*, **34**, 445 (1934).
8. Osborne, D. J. Thesis, Univ. London (1950).

Crystalline α-Lipoic Acid: A Catalytic Agent Associated with Pyruvate Dehydrogenase

Lester J. Reed, Betty G. DeBusk, I. C. Gunsalus, and Carl S. Hornberger, Jr.

Biochemical Institute* and *Department of Chemistry*, *University of Texas*, and *Clayton Foundation for Research, Austin, Texas*; and *Department of Bacteriology*, *University of Illinois, Urbana

A crystalline compound which in catalytic amounts can replace the growth-stimulating activity of acetate for certain lactic acid bacteria, and which is required for the oxidative decarboxylation of pyruvate by these bacteria, has been prepared from liver.

The stimulatory effect of acetate on growth of lactic acid bacteria was first demonstrated by Snell *et al.* in 1937 (*1*). Guirard *et al.* (*2*) in 1946 reported that some biological preparations contain substance(s) which replace acetate as a growth stimulant for several lactic acid bacteria. The concentration of the acetate-replacing factor(s) was undertaken by these investigators, continued by Getzendaner (*3*), and then by one of the present authors (LJR) and his collaborators.

In 1947 O'Kane and Gunsalus reported (*4*) that an unidentified factor, designated pyruvate oxidase factor (*5*), is required for pyruvate oxidation and dismutation by *Streptococcus faecalis*. Further details of their work were presented at the Gordon Research Conferences at New London in 1949.

TABLE 1

X-Ray Characterization* of Crystalline α-Lipoic Acid

"*d*" Interplanar spacings	I/I° Relative intensity
5.30	.10
4.82	1.00
4.52	.10
4.24	.10
4.04	.80
3.85	.50
3.53	.10
2.99	.10

* The data were obtained on a 114-ml Norelco powder camera using Cu Kα radiation. The sample was mounted in a Pariodion capillary. Those lines possessing less than 10% of the intensity of the strongest band have been omitted.

In 1949 Snell and Broquist reported (*6*) that concentrates of the pyruvate oxidase factor and of "protogen," an essential growth factor for *Tetrahymena geleii* described by Stokstad *et al.* (*7*) were very active in promoting growth of *Lactobacillus casei* in the absence of acetate.

In the fall of 1950 a collaborative program was undertaken by the present authors and Eli Lilly and Company.[1] This work has led to the obtaining of a crystalline compound from processed insoluble liver residues, which is highly active for the growth of *Streptococcus lactis* in the absence of acetate and as an activator of the apo-pyruvate dehydrogenase of *S. faecalis*. This compound is being called α-lipoic acid.[2] The potency of crystalline α-lipoic acid is about 250,000 pyruvate oxidase factor units/mg.[3] In the *S. lactis* assay it possesses about 15,000,000 acetate units/mg;[3] i.e., 1.7×10^{-6} μg/ml of culture medium is capable of supporting half-maximal growth in the absence of acetate.

α-Lipoic acid is very soluble in organic solvents, but only sparingly soluble in water. This compound, as obtained, crystallized in the form of platelets possessing a faint yellow tinge, which melted on the microstage at 47.5°–48.5°. It is an acidic substance possessing a pKa of 4.7. The x-ray diffraction data of the crystalline product are presented in Table 1.

[1] The authors are indebted to Eli Lilly and Company for supplying liver concentrates and for measuring the physical constants reported for the crystalline product.

[2] The name lipoic acid is derived from the fact that the compound is highly soluble in fat solvents, is acidic, and is involved, through oxidative decarboxylation of pyruvate, in the formation of acetate, a precursor of fatty acids. The crystalline compound reported in this paper is designated as α-lipoic acid to indicate that it is the first member to be obtained of a series of chemically related substances which possess acetate-replacing and pyruvate oxidase factor activity. The terms acetate-replacing factor and pyruvate oxidase factor were used previously to indicate biological activity and not to denote specific compounds.

[3] The details of these assays will be reported elsewhere. One pyruvate oxidase unit is equivalent to the manometric response produced by 1 mg yeast extract. One acetate unit is equivalent to the growth response produced by 1 mg sodium acetate.

The fact that materials possessing the biological activity of α-lipoic acid can be recovered from diverse biological sources, coupled with the fact that α-lipoic acid has extremely high biological activity and possesses a catalytic role (in the oxidative decarboxylation of pyruvate), suggests that it is a new member of the family of B vitamins. Further research is in progress on its composition, structure, and biological activity.

References

1. SNELL, E. E., TATUM, E. L., and PETERSON, W. H. *J. Bact.*, **33**, 207 (1937).
2. GUIRARD, B. M., SNELL, E. E., and WILLIAMS, R. J. *Arch. Biochem.*, **9**, 381 (1946).
3. GETZENDANER, M. E. The Concentration of an Acetate-replacing Factor. Thesis, Univ. Texas (1949).
4. O'KANE, D. J., and GUNSALUS, I. C. *J. Bact.*, **54**, 20 (1947).
5. *Ibid.*, **56**, 499 (1948).
6. SNELL, E. E., and BROQUIST, H. P. *Arch. Biochem.*, **23**, 326 (1949).
7. STOKSTAD, E. L. R., *et al. Arch. Biochem.*, **20**, 75 (1949).

The Green Pigment and Physiology of Guard Cells

R. O. Freeland

Department of Botany,
Northwestern University, Evanston, Illinois

There is widespread uncertainty in the minds of botanists as to the composition and physiology of the green pigment located in plastids in the guard cells of most leafy plants. Sayre (*1*) concluded from his extensive study of the physiology of the stomates of *Rumex patientia* that the plastids in the guard cells are different structurally, physiologically, and genetically from the chloroplasts of the mesophyll cells. Using microchemical methods, he was unable to obtain a positive test for chlorophyll in the plastids of guard cells but stated that there is no conclusive proof that it is not chlorophyll, because of the extreme difficulty of making the test upon such small bodies.

The present paper deals with two problems related to guard cells, the determination of the absorption spectrum of the green pigments, and a test for photosynthesis in them.

After examining many plants, the leaves of *Hymenocallis littoralis,* Salisb., were found to be most suitable for investigation. In this species, large pieces of epidermis can be stripped from the leaves, and the presence of a heavy cuticle renders it relatively easy to smooth and straighten them for cleaning and inspection. The latter is important because freshly stripped pieces of epidermis were always found to have some fragments of mesophyll adhering to them. Each piece of epidermis, after being mounted on a glass plate, was scraped on the inner surface with a safety razor blade and then scrubbed with a soft bristle brush and water until it was free of all adhering cells and plastids from the mesophyll. Each strip of tissue was inspected under a microscope and if satisfactory was placed in a darkened test tube of cold acetone to which a pinch of $CaCO_3$ had been added. This procedure was continued at intervals over a period of a month or more until a 30-ml test tube full of loosely packed epidermal tissue was accumulated.

The plant material, plus a little quartz sand, was ground in the acetone with a mortar and pestle. After filtering, the acetone solution was light-green in color. The acetone extract was added to 50 ml of a petroleum ether-acetone mixture (10:1) in a separatory funnel. Gentle rotation and the addition of a small quantity of water brought about separation of the two solvent phases. The water-acetone layer was discarded, and the remaining petroleum ether was washed repeatedly with water. Then enough benzene was added to the petroleum ether to give a solution composed of 9 parts petroleum ether to 1 part benzene. This solution, containing the pigments, was passed, by gravity flow, through an adsorption column 1.5 cm in diameter, consisting from top to bottom of 20 cm powdered sugar, 5 cm $CaCO_3$, and 5 cm alumina. Finally, the column was treated with 10% benzene in petroleum ether in an attempt to resolve any possible components of the adsorbed pigment in a well-defined chromatogram. Only one pigment layer, blue-green in color, was visible in the column. After drying the column by suction with air, this layer was removed. The pigment was eluted from the sugar with a 1:1 solution of methanol and ethyl ether. The extract was freed of methanol by repeated water washing in a separatory funnel. The resulting ethyl ether containing the green pigment was studied immediately in a Coleman Junior spectrophotometer. The data on light absorption in terms of density are presented in Fig. 1. The entire experiment, from the collection of

FIG. 1. Absorption data for ethyl ether solutions of the green pigments extracted from the leaves of *Hymenocallis*. *P*, pigment from the guard cells: *A* and *B*, chlorophylls *a* and *b*, respectively, from the mesophyll. (Density = − log Transmittance.)

epidermal tissue to pigment analysis, has been repeated with the same qualitative results. For comparative purposes the pigments were extracted from the mesophyll tissues, separated, and studied in the same way. The chromatogram for the pigments from the mesophyll showed the usual distribution from top to bottom of the adsorption column of xanthophyll,

chlorophyll *b*, chlorophyll *a*, and carotin. Absorption data for chlorophylls *a* and *b* are presented in Fig. 1.

Absorption spectra of chloroplast pigments in various physical states and in different solvents are well known. The principal absorption maxima for chlorophylls *a* and *b* in ethyl ether are 430 and 660 mμ, and 455 and 642 mμ, respectively (*2*). The chlorophylls from the mesophyll of *Hymenocallis* were found to have such absorption maxima (Fig. 1) and were unquestionably chlorophylls *a* and *b*. The green pigment isolated from the guard cells of the epidermal tissue has two absorption maxima, at 420–430 mμ and 660–670 mμ, indicating that it is predominantly chlorophyll *a*. The breadth of the absorption band below 475 mμ for the guard cell pigment, and the lack of complete conformity in shape between the density curves for the guard cell pigment and chlorophyll *a*, suggest the possibility that traces of either carotinoids or chlorophyll *b* or both were associated with the green pigment extracted from the guard cells. Although the developed chromatogram of the guard cell pigments showed only one visible band, it should be noted that the quantity of pigment being handled was quite small.

Numerous and varied experiments have been performed using luminous bacteria, *Photobacterium fischeri*, which glow in the presence of oxygen, to test for photosynthesis in guard cells. Each experiment consisted of three groups of culture vessels, test tubes, or van Tieghem cells, all of which contained active bacteria. One set of cultures contained in addition several strips of epidermal tissue; one set contained a small piece of *Elodea, Anacharis canadensis;* and one set was left without any chlorophyllous tissue. The circumambient solution consisted either of the liquid culture in which the bacteria were growing or of a solution of 0.1% $KHCO_3$, to which a high concentration of the bacteria had been added. After the culture vessels were sealed to exclude further access to air, they were placed in a darkroom, and the fluid in them was deoxygenated either by passing a slow stream of nitrogen gas through each or by the organisms themselves. After deoxygenation was completed, as indicated by the cessation of light from the cultures, the test material was exposed to light for various periods of time and then observed as quickly as possible in the dark. Exposures to several intensities of both natural and artificial (Mazda) light were tried. In every experiment the check cultures, containing *Elodea*, were luminous after exposure to light and the cultures without green tissues were not. Evolution of oxygen in the test cultures containing strips of epidermal tissue, as indicated by the bacteria becoming luminous, was not observed. In brief, all tests for photosynthesis in guard cells by this method were negative.

According to Curtis and Clark (*3*), Alvim through the use of the starch test has obtained evidence that photosynthesis occurs in the guard cells of bean plants. The presence of chlorophyll in the guard cells of *Hymenocallis* suggests but does not prove that photosynthesis takes place in them, since chlorophyllous plants are known which do not carry on photosynthesis (*4*). Failure to demonstrate photosynthesis in guard cells through the use of oxygen-sensitive, luminous bacteria does not prove conclusively that the process does not occur in them, for several reasons. In the cultures containing epidermal tissue there was, relatively, a very small number of green cells in comparison with the total number of nongreen cells. Use of oxygen by the latter may have made it impossible to detect any luminosity of the bacteria in the vicinity of the guard cells even with a microscope. Furthermore, submergence of the epidermal tissue plus the rather drastic treatment to which it was subjected in preparation for the tests may have inhibited photosynthesis.

References

1. Sayre, J. D. *Ohio J. Sci.*, **26**, 233 (1926).
2. Zscheile, F. P. *Botan. Rev.*, **7**, 587 (1941).
3. Curtis, O. F., and Clark, D. G. *Introduction to Plant Physiology*. New York: McGraw-Hill, 2nd (1950).
4. Smith, J. H. C. *J. Chem. Education*, **26**, 631 (1949).

Growth of Human Leukemic Leucocytes *in Vitro* and *in Vivo* as Measured by Uptake of P^{32} in Desoxyribose Nucleic Acid[1, 2]

Edwin E. Osgood, Jonah G. Li,[3] Harold Tivey, Marie L. Duerst, and Arthur J. Seaman

Department of Medicine,
Division of Experimental Medicine,
University of Oregon Medical School, Portland

By determination of uptake rates of radioactive phosphorus (P^{32}) into desoxyribose nucleic acid (DNA), it is possible to obtain a quantitative measure of the rate of formation of human leucocytes in culture (*1*), and by parallel determinations in the cell-donor patient with leukemia, treated with P^{32}, to compare the rate *in vitro* with that of the same population of leukemic cells in the patient. This information is otherwise unobtainable, since leucocyte death is occurring concurrently with cell division, both in the culture and in the patient. The rate of cell formation supplements the information on the rate of cell differentiation, obtainable from total and differential cell counts, and the rate of mitosis obtainable with colchicine. It is the purpose of this paper to present evidence for the initial statement, and to outline briefly the techniques used and the preliminary results.

[1] A preliminary report.

[2] This investigation was supported by grants-in-aid from the Medical Research Foundation of Oregon; the Damon Runyon Cancer Research Fund; the National Cancer Institute, of the National Institutes of Health, USPHS; and the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. The P^{32} used was supplied by Clinton Laboratories and obtained on allocation from the Isotopes Division, United States Atomic Energy Commission.

[3] Present address: Division of Preventive Medicine, University of California School of Medicine, San Francisco.

TABLE 1

PARTITION OF PHOSPHORUS IN TISSUES OF AN ADULT PATIENT WITH LEUKEMIA TREATED FOR 1 YEAR WITH RADIOACTIVE PHOSPHORUS*

Tissue	All non-DNA phosphorus				DNA phosphorus			
	P^{31}	P^{32}	Specific activity	Plasma specific activity (%)	P^{31}	P^{32}	Specific activity	Plasma specific activity (%)
Spleen	162	5.16	0.0414	141	69.1	1.10	0.0159	54
Lymph nodes	189	5.74	.0303	103	123	1.41	.0115	39
Liver	288	8.90	.0309	105	33.2	0.32	.0097	33
Kidney	182	4.82	.0265	90	35.7	0.33	0.0093	32
Skeletal muscle	191	2.50	.0131	45	97	0	—	—
Cartilage	32	0.59	.0183	62	14	0	—	—
Brain, cortex	237	0.90	0.0038	13	31.5	0	—	—

* Tissues were obtained, before embalming, 1 hr after death, which occurred 3 days after the last dose of 0.033 μc P^{32}/g body wt. The tissues were kept iced until DNA extraction. All values above are mg P^{31} or μc P^{32}/100 g wet tissue. Note that adequate P^{32} is available in the muscle, cartilage, and brain and that, despite readily measurable quantities of DNA P^{31}, no DNA P^{32} uptake was found in those tissues in which cell division does not take place.

Marshak's (*2*) pioneer work on phosphorus turnover in liver nuclei suggested that incorporation of P^{32} in DNA occurs principally during mitosis. This view is supported by Hull and Kirk (*3*) and by others cited (*3*). Independent evidence from this laboratory in support of this hypothesis comes from three sources. First, incubation of isolated desoxyribose nucleic acid at 37° C with labeled inorganic phosphate of 10,000 cpm/ml activity showed no significant exchange in 24 hr. Second, control aliquots removed from cultures of leukemic leucocytes and maintained at 4° C showed no DNA P^{32} uptake over periods of 4 days. Third, post-mortem determinations of DNA P^{31} and P^{32} in the tissues of a patient with leukemia, treated with therapeutic doses of P^{32} for a period of 1 year, failed to show any measurable uptake of P^{32} in DNA of brain, skeletal muscle, or cartilage—tissues considered by most anatomists to undergo no cell division in the adult—whereas uptake of P^{32} was evident in the DNA of tissues in which mitotic division is known to occur. The results of this study are summarized in Table 1.

The basic culture techniques were those previously described by Osgood (*1*) with the following modifications. Leucocytes from patients with leukemia were isolated aseptically from venous blood, using the phytohemagglutinin technique of Li and Osgood (*4*). The culture medium used is shown in Table 2. Culture vessels were Pyrex aspirator bottles, 4-liter size for 1-liter cultures, or of a similar size ratio for smaller cultures. These bottles were autoclaved with cotton plugs in their tops and rubber vaccine-vial caps (size 1A) wired on the tubing outlets. Cultures of 4,000–10,000 cells/mm^3 were made by aseptically introducing the cells through the tubing outlet into the medium; after mixing by gentle rotation, the vessels were placed in the 37° C incubator on their sides, with the tubing outlets upward.

TABLE 2

COMPOSITION OF THE CULTURE MEDIUM

Balanced salt solution			
NaCl	8.00 g	$Na_2HPO_4 \cdot 2H_2O$	0.15 g
KCl	0.37	KH_2PO_4	0.03
$CaCl_2$ (anhydrous)	0.17	$MgSO_4 \cdot 7H_2O$	0.07
$MgCl_2 \cdot 6H_2O$	0.21	Dextrose	2.00

Dissolve in the order given and add distilled H_2O to 1 liter. Sterilize by Seitz filtration. Prepare medium by mixing 1 part aseptically collected human serum with 2 parts balanced salt solution and adding 100,000 units/liter of K salt penicillin G. Adjust pH to 7.60 with sterile *N*/1 NaOH or *N*/1 HCl.

After mixing thoroughly, samples were withdrawn daily for total and differential cell counts and pH determinations. The pH was adjusted to approximately 7.6 daily by the use of sterile *N*/1 NaOH, but the medium was not changed. At intervals of 2 days or more, a sufficient quantity of the mixed culture was aseptically withdrawn, by the use of syringes, to yield about ½ billion granulocytes or 1 billion lymphocytes. The cells were harvested by centrifugation, and, after determination of the packed cell volume, DNA was determined by a modification of the Schmidt-Thannhauser (*5*) method, in which the packed cells were dissolved directly in the *M* KOH. The precipitate that resulted from this technique was washed six times with ice-cold 0.5 *N* HCl and 5% trichloracetic acid, redissolved, ashed, and its P content determined by the method of Fiske and SubbaRow. An aliquot of the solution used for the determination of DNA phosphorus was plated out and the radioactivity determined as described by Tivey and Osgood (*6*). Total P^{31} and P^{32} were determined on aliquots of the medium.

When possible, parallel determinations of P^{32} uptake in DNA were made in the patient and in culture. The P^{32} level in the plasma of the patient depends upon the initial dose needed by that particular patient (*7*), and to obtain equivalent radiation effects the P^{32} content of the culture was made equal to the plasma level in the patient, as calculated by the formulas described by Osgood and Tivey (*8*). Daily injections of one tenth or less of the initial dose served to maintain the plasma P^{32} level of the patient essentially constant. Since the dose of P^{32} injected varies from

one patient to another, all final results were made comparable by expressing DNA P^{32} in terms of percentage of specific activity (total P^{32} in μc/100 ml ÷ total P^{31} in mg/100 ml) of the plasma or of the culture medium.

The results of a culture of cells from a patient with chronic granulocytic leukemia are shown in Fig. 1; those from a patient with acute lymphocytic leukemia in Fig. 2. Data on 5 uptake cultures of cells from patients with chronic granulocytic leukemia are shown in Fig. 3. Obviously, the rate at which P^{32}

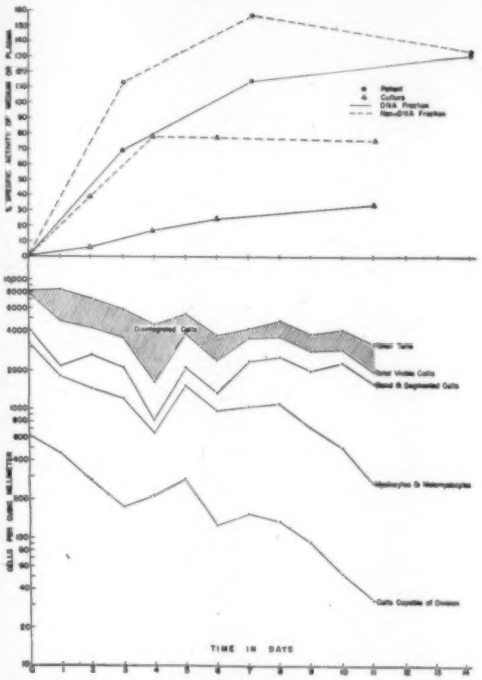

FIG. 1. Culture of cells from the blood of a patient with chronic granulocytic leukemia. The DNA uptake rate *in vitro* is approximately one fifth that in the patient, but the large fraction of distintegrated cells present in culture, if "unlabeled by P^{32}," would tend to decrease the observed uptake markedly. "Cells Capable of Division" include blasts, progranulocytes, and early myelocytes.

disappears from the DNA of cells cultured in P^{32}-free medium from a patient who had been previously treated with P^{32} should be an equally good measure of the rate of cell death and autolysis and, if the cell count remains constant, of the rate of new cell formation. However, since P^{32} cannot be removed instantly from extracellular fluid, this technique cannot be employed *in vivo*.

Three attempts to culture the cells of patients with chronic lymphocytic leukemia have failed to show significant uptake of P^{32} in DNA over periods of time up to 10 days, although uptake of P^{32} in the non-DNA fractions occurred. This result is to be

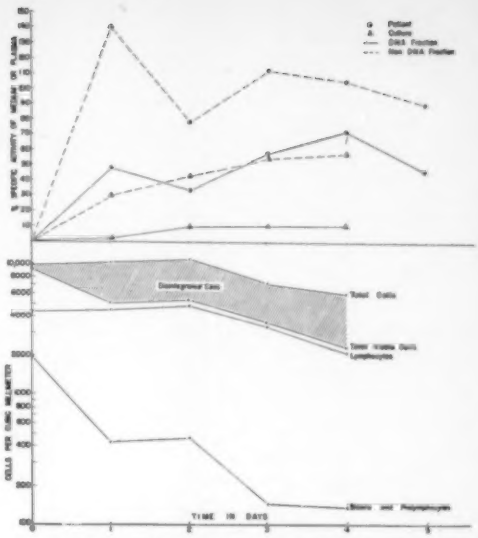

FIG. 2. Culture of cells from a patient with acute lymphocytic leukemia. DNA uptake of P^{32} in culture is small, though significant, and in marked contrast to the negligible uptake observed in cultures of cells from patients with chronic lymphocytic leukemia.

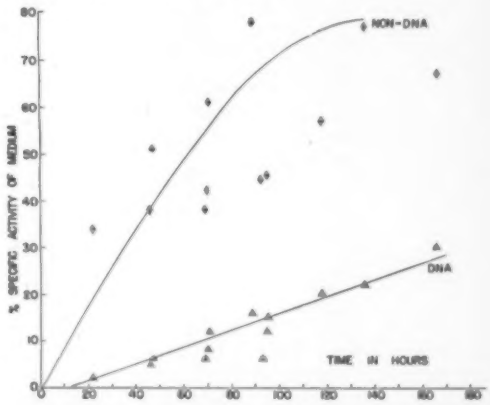

FIG. 3. Results of 5 cultures of cells from patients with chronic granulocytic leukemia. Uptake rates of P^{32} in DNA (indicative of cell division) of approximately 1% of medium specific activity per 10 hr may be noted. Non-DNA P^{32} uptakes are much faster. The sole P^{32} in the medium is introduced as inorganic P. Serum organic compounds totaling approximately 50% of all P present were initially unlabeled. If these latter compounds are also used in new DNA formation, the indicated uptake rate, estimated by DNA P^{32} specific activity ratio to that of the medium, is much lower than that which actually takes place.

expected in view of the uniformly slow rate of new DNA formation observed *in vivo*.

The slope of the uptake curve of P^{32} in DNA in the cultures of cells from patients with chronic granulocytic leukemia indicates new cell formation of at least 20% of that observed in the patient. There

are several reasons why the growth of leucocytes in these cultures would not be expected to equal that occurring *in vivo*. Among these are the fact that the cultures were suboptimum as compared to the best culture technique, in containing too many cells per unit volume, in depth of layer over most of the cells, which regulates the O_2 tension, in composition of the medium, and in that no change of medium was made. Furthermore, there is no assurance that a sample of leukemic blood contains the same proportion of undifferentiated cells capable of division as is present in the total blood-forming tissue of the leukemic patient.

The data presented appear to demonstrate that growth of leucocytes from patients with chronic granulocytic or acute lymphocytic leukemias takes place *in vitro*, and that the rate of new cell formation can be estimated by the rate of uptake of P^{32} in the DNA fraction, either *in vivo* or *in vitro*.

References

1. OSGOOD, E. E. In *A Symposium on the Blood and Blood-forming Organs*. Madison: Univ. Wis. Press, 219 (1939).
2. MARSHAK, A. *J. Gen. Physiol.*, **25**, 275 (1941).
3. HULL, W., and KIRK, P. L. *Ibid.*, **33**, 335 (1950).
4. LI, J. G., and OSGOOD, E. E. *Blood*, **4**, 670 (1949).
5. SCHMIDT, G., and THANNHAUSER, S. J. *J. Biol. Chem.*, **161**, 83 (1945).
6. TIVEY, H., and OSGOOD, E. E. *Cancer*, **3**, 992 (1950).
7. OSGOOD, E. E. *Arch. Internal Med.*, **87**, 329 (1951).
8. OSGOOD, E. E., and TIVEY, H. *Cancer*, **3**, 1003 (1950).

D-Catechol and Antihistaminogenesis

Saul Malkiel and Margaret D. Werle

Allergy Research Laboratory, Department of Medicine, Northwestern University Medical School, Chicago, Illinois

It has been agreed (*1, 2*) that certain flavonoid compounds have been found capable of the *in vitro* inhibition of an enzyme possessing the capacity of decarboxylating histidine to histamine (*3–5*). Of these, D-catechol was reported as being the most active (*1*). However, conflicting reports (*6–12*) have appeared regarding the usefulness of the flavonoids in the *in vivo* inhibition, as evidenced by experimental sensitization phenomena. Thus, Moss, Beiler, and Martin (*12*) have indicated that D-catechol protected guinea pigs against the anaphylactic shock reaction in contradistinction to the findings of Clark and Mackay (*11*), who confirmed the reported (*7, 9, 10*) negligible effect of the flavonoids. Because of the far-reaching potentialities of an antihistaminogenic compound in clinical allergic diseases, wherein the manifestations are often the result of histamine release, it was felt desirable to repeat, verify, and amplify the findings.

In vitro inhibition. The procedure of Beiler, Brendel, Graff, and Martin (*2*) was followed essentially as described. Reaction mixtures of kidney extract, D-catechol, and L-histidine were prepared. Three control mixtures were simultaneously carried out, each containing only two of the constituents. Isolation of the histamine formed in the reaction was carried out essentially according to the method of McIntire, Roth, and Shaw (*13*) as adapted for chromatographic identification by Urbach (*14, 15*). Histamine, when present in amounts exceeding 2–3 mg, makes its appearance on the filter paper strips as a red band at an R_F value of approximately .56.

In vivo inhibition. Two techniques were utilized to actively sensitize guinea pigs.

Series 1. Seven guinea pigs were sensitized according to the procedure described by Raiman, Later, and Necheles (*8*) as advocated by Moss, Beiler, and Martin (*12*). For seven days prior to sensitization each guinea pig received 2 mg D-catechol daily, given intraperitoneally (i.p.). On the eighth day, in addition to the D-catechol, 0.25 ml horse serum was administered i.p. For 11 subsequent days the animals were each given 2 mg D-catechol i.p. daily. On the twelfth day, the animals were challenged by the intravenous (i.v.) administration of 0.05 ml/100 g body weight of horse serum. Ten control guinea pigs were each sensitized by the i.p. injection of 1 ml of a 1 : 4 dilution of horse serum in physiological saline, but received no D-catechol. On the thirteenth day challenge was accomplished by the i.v. administration of 0.2 ml

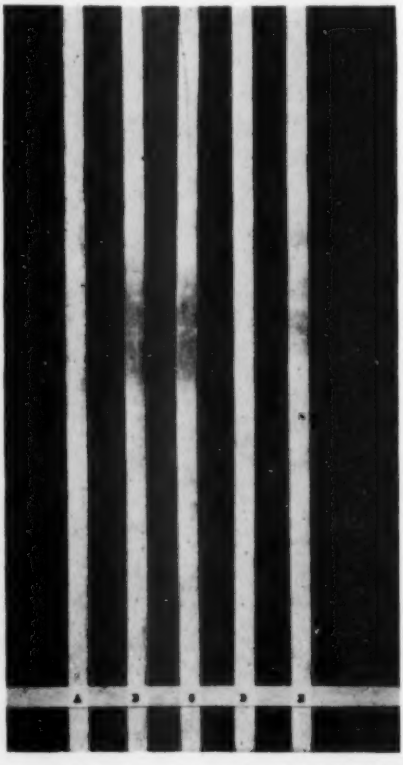

FIG. 1. *A*, kidney extract + D-catechol; *B* and *C*, kidney extract + L-histidine; *D*, kidney extract + D-catechol + L-histidine; *E*, D-catechol + L-histidine.

horse serum (diluted 1:4)/100 g of body weight.

Series 2. Ten guinea pigs were each pretreated by the i.p. injection of 2 mg D-catechol for 4 days. On the fifth day they each received, in addition, 1 ml of a 1 : 10 dilution of horse serum given subcutaneously. D-Catechol was continued for 13 subsequent days. On the fourteenth day, each animal was challenged by the i.v. injection of 1 ml of horse serum. A simultaneous control group was treated identically except for the D-catechol administration.

In vitro inhibition. D-Catechol effectively inhibited the action of the histidine decarboxylase present in the guinea pig kidney extract. The reaction mixture without the D-catechol showed appreciable amounts of liberated histamine. The other controls were negative. The chromatogram is shown in Fig. 1.

In vivo inhibition. Series 1. As Table 1 indicates,

TABLE 1

RESULTS OF ANAPHYLACTIC CHALLENGE OF ACTIVELY SENSITIZED (TO HORSE SERUM) GUINEA PIGS TREATED WITH D-CATECHOL

Guinea pig No.	Series 1		Series 2	
	Control	Treated	Control	Treated
1	++++	++++	++++	++++
2	++++	+++	++++	++++
3	++++	+++	++++	++++
4	+++	++	++++	++++
5	+++	++	++++	++++
6	+++	++	++++	++++
7	+++	++	++++	++++
8	++		++++	++++
9	++		++++	++++
10	++		++++	++++

++++ Died.
+++ Markedly severe symptoms; collapse with eventual recovery.
++ Severe symptoms; marked respiratory distress.

3 of the control group of guinea pigs died in shock, 4 survived markedly severe anaphylactic symptoms, and 3 suffered severe reactions. Of the test group, 1 died in anaphylactic shock and 6 survived. Of the survivors 2 suffered markedly severe and 4 severe symptoms.

Series 2. All control and test animals died in anaphylactic shock.

There seems to be little doubt that D-catechol can inhibit the action of tissue histidine decarboxylase. However, these results confirm those of others (*7, 9–11*) that the flavonoid is without an appreciable *in vivo* action on the enzyme. Since in our hands the sensitization technique of Raiman, Later, and Necheles failed to produce uniformly fatal results in the control group of animals, we considered the results obtained in the treated group as equivocal. For this reason a sensitization procedure was adopted in which an LD_{100} was employed. The treated animals in this group showed no resistance to the challenge. If D-catechol does have an *in vivo* inhibiting action on tissue histidine decarboxylase, the results suggest either that anaphylactic symptoms are produced by some mechanism other than histamine release or that inhibition is not complete and at least a sufficient amount of histamine is formed to account for the symptoms. However, the severity of the reactions seems to indicate that D-catechol plays but a negligible role in preventing anaphylactic shock in the guinea pig, which presumably results from histamine release.

References

1. MARTIN, G. J., *et al. Arch. Biochem.*, **21**, 177 (1949).
2. BEILER, J. M., *et al. J. Am. Pharm. Assoc.*, **38**, 315 (1949).
3. WERLE, E. *Biochem. Z.*, **288**, 292 (1932).
4. WERLE, E., and HERRMANN, H. *Ibid.*, **291**, 105 (1937).
5. HOLTZ, P., and HEISE, R. *Arch. exptl. Path. Pharmakol.*, **186**, 377 (1937).
6. HULLSTRUNG, H., and HACK, K. *Z. Immunitätsforsch.*, **100**, 393 (1941).
7. WILSON, R. H., MORTAROTTI, T. G., and DE EDS, F. J. *Pharmacol. Exptl. Therap.*, **90**, 120 (1947).
8. RAIMAN, R. J., LATER, E. R., and NECHELES, H. *Science*, **106**, 368 (1947).
9. ROTH, L. W., and SHEPPERD, I. M. *Science*, **108**, 410 (1948).
10. ARBESMAN, E. E., and NETER, E. *J. Allergy*, **20**, 80 (1949).
11. CLARK, W. G., and MACKAY, E. M. *Ibid.*, **21**, 133 (1950).
12. MOSS, J. N., BEILER, J. M., and MARTIN, G. J. *Science*, **112**, 16 (1950).
13. MCINTIRE, F. C., ROTH, L. W., and SHAW, J. L. *J. Biol. Chem.*, **170**, 537 (1947).
14. URBACH, K. F. *Proc. Soc. Exptl. Biol. Med.*, **70**, 146 (1949).
15. *Ibid.*, **72**, 626 (1949).

Counting of Radioactivity in Liquid Samples

R. F. Goddu and L. B. Rogers[1]

Department of Chemistry and
Laboratory for Nuclear Science and Engineering,
Massachusetts Institute of Technology, Cambridge

During the recent work using the counting technique proposed by Freedman and Hume (*1*) for liquid samples, two pitfalls were noted when the samples were not counted immediately. In the present study, aluminum cups were carefully coated with Chem-Lac Lacquer 117-2 (Chem-Laq Products, Inc., Cambridge, Mass.) and allowed to dry. The samples counted contained 10^{-3} *M* silver as carrier and 7.5-day Ag^{111} as tracer in 0.8 *M* potassium thiocyanate solution. The surface of the sample was coated with thinned Chem-Lac Lacquer, as described by Freedman and Hume, and allowed to dry. At least 10,000 counts were taken on each sample to cut the statistical error of counting to 1%.

When 1-ml samples were counted in the coated aluminum cups, appreciable plating-out of silver took place within 24 hr, as evidenced by the substantial decrease in activity in Set 1, Table 1. The plating was also visible to the naked eye. After 2 more days, another complication was evidenced by a substantial increase in count, which more than compensated for the decrease due to plating.

Studies of this increase in counting rate were carried out in lacquered glass cups to avoid changes

[1] The authors are indebted to the Atomic Energy Commission for support of this work.

TABLE 1

CHANGE IN COUNTING RATE WITH AGE OF SAMPLE, CORRECTED FOR DECAY

Set	Description of sample	Relative counting rate on successive days						
		0	1	2	3	4	5	7
1	1 ml of solution in Al cup kept in air (av of 2)	100	73	—	—	82	—	—
2	1 ml of solution in glass cup kept in air (av of 3)	100	106	109	111	115	—	125
3	1 ml of solution in glass cups kept in hygrostat over 1 *M* KSCN (av of 4)	100	104	105	104	104	—	106
4	5 ml of solution in glass beakers kept in air (av of 2)	100	98	98	100	—	—	—
5	1 ml of solution in glass cups kept in hygrostat over water (av of 7)	100	100	100	—	—	100	99

TABLE 2

RELATIVE COUNT CORRECTED FOR DECAY AND FOR DECREASE IN VOLUME

Set	Description of sample	Relative counting rate on successive days					
		0	1	2	3	4	7
2	1 ml of sample in glass cups kept in air (av of 3)	100	101	99	99	99	92
3	1-ml samples in glass cups kept in hygrostat over 1 *M* KSCN (av of 4)	100	102	103	101	101	102

caused by plating. Adsorption onto the lacquered surface was not thought to be significant, because of the presence of carrier and complexing agent, an assumption substantiated by the experimental results described below. When samples were weighed immediately before or after they were counted, an observed count could be corrected by a factor that took into account the increase in concentration resulting from loss of solvent through the dry film of lacquer. Set 2 in Tables 1 and 2 illustrates typical results before and after multiplying by a correction factor which is the percentage of original volume remaining at the time the sample was counted. As one might expect, the evaporation can be eliminated by using a hygrostat, as shown by Set 5. Occasionally, small droplets of water condensed on top of the film, but they can be removed easily without damage to the film by using an absorbent paper tissue. Ordinarily, the change in weight of a cup having a surface area of approximately 3.5 cm^2 was about -30 mg/day in the open air, -6 mg/day over 1 *M* potassium thiocyanate, and ± 1 mg/day over water. One can also decrease the evaporation error by using a large volume of sample without changing the surface area, so that the relative loss in weight is insignificant, as illustrated by Set 4.

These studies point out that in spite of careful coating of a metal container the plating of a more noble metal onto a less noble metal is very probable. More important is the fact that either the counting of liquid samples must be done within a few hours after preparation of the sample or the loss of solvent eliminated by placing the samples in a hygrostat after the lacquer film has dried. For best results, a correction should be applied for changes in the volume of the sample. Use of a hygrostat appeared to increase the average lifetime of the lacquer films from about 10 days in open air to about 3 weeks.

Reference

1. FREEDMAN, A. J., and HUME, D. N. *Science*, **112**, 461 (1950).

A Volumetric Microrespirometer for Studies of Tissue Metabolism[1]

Reidar Wennesland[2, 3]

Physiology Department,
Stanford University, Stanford, California

A microrespirometer has been developed for studies of metabolism in small animals, tissues, cell suspensions, etc. It is based upon models of volumetric respirometers as constructed by Winterstein (*1, 2*), Scholander (*3, 4*), and Wennesland (*5*). From Scholander (*4*) has been adopted the use of a plastic block into which a V-shaped manometer has been drilled, connecting the respiration chamber with the compensating vessel.

New features are the inclusion in the plastic block of a chamber for oxygen replacement, and a new measuring device for the gas exchange. The latter has also been developed into a measuring and delivery burette for regular laboratory use (unpublished).

The apparatus is a constant pressure respirometer, maintaining the principal features of Winterstein's original model. The gases are kept under constant temperature and pressure, and the changes in volume are read directly. The theory is thus very simple: no vessel constants need be determined or calculated. The

[1] This work was supported by a Norwegian government scholarship and in part by contract N-6 onr 251-XIV between Stanford University and the Office of Naval Research.

[2] I wish to express my gratitude to John H. Lawrence, of the Donner Laboratory, University of California, Berkeley, and his staff for support and hospitality.

[3] Present address: The Permanente Hospital, Oakland, Calif.

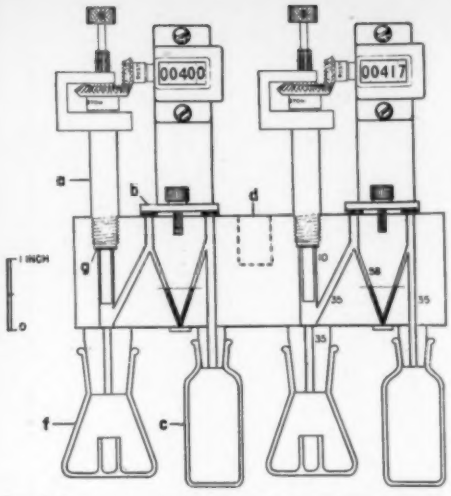

FIG. 1. One manometer block with two respirometer units.

only part to be calibrated is the volumetric device, which is uniform for all sets. Vessels of different types and sizes can be applied, and media of different quantity and composition used, without recalibrating the apparatus. The manometer necessary for adjusting the constant pressure is closed and connected to a compensating chamber, which makes it insensitive to changes in barometric pressure and humidity during the experiment, and much less sensitive to changes in the environmental temperature than an uncompensated system.

The apparatus (Fig. 1) consists of the following parts: respiration chamber; compensating vessel; Plexiglass manometer block, which also contains the oxygen delivery chamber; delivery and measuring device for oxygen; and mounting and shaking device.

The conventional Warburg flasks with one side arm and center well may be used for the respiration chamber (Fig. 1, *f*). I have made such flasks easily and cheaply from Plexiglass (Fig. 2 *A, f*).

For compensating vessels small bottles (Fig. 1, *c*) with standard ground glass stoppers (e.g., A. H. Thomas, Cat. No. 2232), of approximately the same volume as the Warburg flasks, may be employed. It is preferable to make the compensating chambers from Plexiglass. Their openings can be provided with screw threads to fit a threaded plug instead of the tapered one seen in Fig. 1. Plexiglass Warburg flasks can also be made with a threaded connection. If wanted, the threaded plug is converted into a tapered one by an adapter. The advantage of the threaded connection is the tight fit, which eliminates the need for such tightening devices as springs, rubber bands, etc., generally used to secure the tapered attachments.

The Plexiglass manometer block adapted for two respirometers is shown in Fig. 1. The figures to the right of the bores show the scale number of the drills used. After the apparatus has been thermoequilibrated, the manometer openings are closed simultaneously by a crossbar with two neoprene disks (Fig. 1, *b*). The bar is loosened and fastened by a binding post (Eby junior).

For the delivery and measurement of oxygen a simplified micrometer device is used (Fig. 1, *a*): the plunger and micrometer screw are made in one piece from a polished stainless steel rod of 3/16-in. diameter. The micrometer barrel can be made of Plexiglass or stainless steel. It is attached to the upper part of the oxygen chamber by a screw thread, and has a double gasket (*g*) of neoprene and vulcanized fiber material to give an airtight fit. The screw movement of the plunger is transferred through a set of bevel gears (Boston gear, Cat. No. G 479) to a commercial revolution counter (Veeder Roth square case revolution counter, Cat. No. A 114135). By choosing a micrometer screw thread of 32 to the inch, a gear ratio of 2/1 and the counter type mentioned, which records 10 units for each counter axis revolution, one inch displacement of the plunger gives 640 reading units ($32 \times 2 \times 10$). Each counter unit thus represents a volume displacement of .707 µl.

The mounting and shaking device is shown in Fig. 2. (I am indebted to Professor J. M. Crismon, of the Physiology Department at Stanford University, for

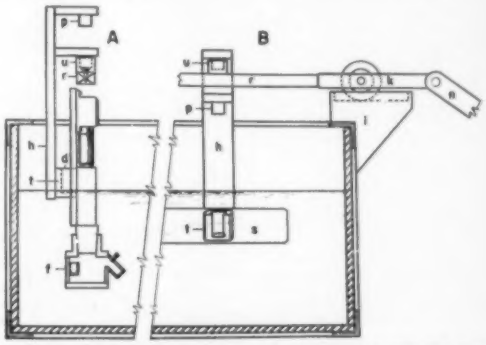

FIG. 2. *A*, side view of mounting device and shaking rod with one respirometer attached, suspended in the upper position with manometer bores above the water surface. *B*, right end of the same without respirometer, suspended in the deepest position. (Front view.)

valuable suggestions.) The respirometer blocks can be attached individually to the shaking rod by a double suspension hook of Plexiglass (Fig. 2 *A, h*), or the receptacles (*t*) can all be cemented to a strip of Plexiglass (*s*), each end of which is suspended from the shaking rod by similar hooks (Fig. 2 *B, h*). A modified dovetail arrangement is used for the mounting. The male parts (Figs. 1 and 2 *A, d*) are made from pieces of ½-in. Plexiglass rod, of which a segment is milled off to provide a face for cementing it to the back of the manometer block. The receptacles (Fig. 2, *t*) are made from a ¾-in. rod, into which a hole of ½-in. diameter is drilled, and 2 parallel faces

milled off, one corresponding to the segment milled off from the male part, the other for cementing the receptacles to the mounting strip. The suspension hooks rest by small plugs (p) in cups (u) mounted on top of the aluminum shaking rod (r). The rod is extended across the water bath, and has a small ball-bearing carriage (k) at each end, which rolls in a shallow groove of a rig (i) attached to the edges of the water bath. It is driven by a crank (n). The amplitude and rate of shaking are of the same order as with the conventional Warburg apparatus.

The only calibration necessary is to measure the diameter of the plungers by means of a commercial micrometer caliper. For very accurate work this should be done at the experimental temperature. The feed of the micrometer screw should be checked against a micrometer caliper head.

All parts are greased with a chemically neutral grease, such as Nevastane heavy X (Keystone). The compensating vessels should contain a few drops of water. With a long syringe needle the manometers are filled to the level line with the manometer fluid; e.g., water containing a little detergent and some dye such as T 1824, or kerosene with a little Sudan IV. The tissue or other respiring material is placed in the respiration chamber, which contains the medium and in the center well about 0.2 ml 5% KOH. The chamber can be flushed with oxygen through the side arm. After the side arm has been closed, the mounting strip with the respirometers is attached to the shaking rod in the upper position (Fig. 2 *A*) with the manometer openings above the water. It should be shaken 10–15 min for thermoequilibration. The manometer openings are closed, and the mounting device is moved to its deepest position (Fig. 2 *B*), so that all gas volumes are under water. After shaking another 10 min, the manometers are adjusted to the level line, and the initial reading is taken. At regular intervals the manometers are brought back to balance and readings are made.

The difference between consecutive readings is the oxygen consumption for that interval, uncorrected for standard temperature and pressure. Each counter unit corresponds to .707 µl. Suppose, in an experiment measuring oxygen consumption, the average difference between readings taken at intervals of 15 min is 32 counter units: The oxygen consumption for that period has been $32 \times .707 = 23.6$ µl of O_2 at the temperature of the experiment, and barometric pressure as observed at the time of the closure of the manometers. The figure is converted into volume O_2NTP per unit of tissue weight per hour in the usual way.

Blank runs have given an oxygen consumption of 1–2 counter units per hour, which is generally negligible.

For further details as to the construction of the respirometer, operation, etc., I refer to the description of the previous model in Umbreit (*5*), and to Peiss and Wennesland (*6*). As the principal features are retained, the experiences of Peiss and Wennesland are directly applicable to the present model: The

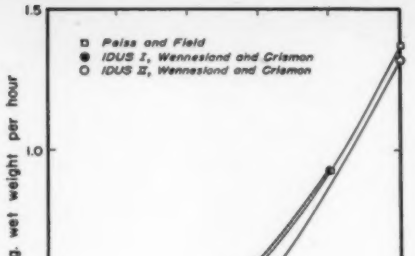

FIG. 3. Oxygen consumption of brain mince of the Golden Orfe (*Idus melanotus*) adapted to 25° C tested at temperatures from 0° C to 25° C. Data from Peiss and Field's work performed with the Warburg apparatus (137 observations) compared to two groups (Idus I and II) tested with the present respirometer (89 observations). O_2 consumption in µl/mg wet wt/hr.

oxygen consumption of rat brain cortex slices was tested at 37.5° C, 3 aliquants by the Warburg method and 3 by the volumetric apparatus. In all, 48 Warburg runs were made and 47 volumetric (one sample lost). The mean Q_{O_2}s were 14.12 and 14.08, respectively. The corresponding standard errors were ± 0.169 and ± 0.171. Statistical analysis showed that the two series did not differ significantly either in respect to the means (Student's t test) or of the variability (Fisher's F test).

The present model has been used for more than a year in an extensive series of studies by Wennesland and Crismon (*7*) of brain tissue metabolism of fish at temperatures between 0° C and 25° C. Figures from two groups of the Golden Orfe (*Idus melanotus*) adapted to 25° C compare favorably with corresponding experiments published recently by Peiss and Field (*8*), who used the Warburg method. In Fig. 3 the curves from the two groups of *Idus* experiments (called Idus I and II) are compared with the corresponding curve drawn from the figures in Peiss and Field's paper.

The range of the apparatus can be extended downward and upward by changing the dimensions. I have made respirometers with a sensitivity of 0.05 µl, and a large modification which allows a total gas displacement of about 6 ml divided into 1,280 counter units. Because of the size of the chamber of the latter construction the manometer block and compensation chamber were cemented on top of a circular base plate. The respiration chamber was attached underneath by a screw clamp device.

References

1. WINTERSTEIN, H. *Biochem. Z.*, **46**, 440 (1912).
2. ———. *Z. Biol. Tech. Methodik*, **3**, 246 (1913).

3. SCHOLANDER, P. F. *Rev. Sci. Instruments*, **13**, 32 (1942).
4. *Ibid.*, **20**, 885 (1949).
5. WENNESLAND, R. In W. W. Umbreit, R. H. Burris, and J. F. Stauffer (Eds.), *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*. Minneapolis: Burgess Pub. (1948).
6. PEISS, C. N., and WENNESLAND, R. *Federation Proc.*, **8**, 126 (1949).
7. WENNESLAND, R., and CRISMON, J. M. Unpublished data.
8. PEISS, C. N., and FIELD, J. *Biol. Bull.*, **99**, 213 (1950).

A New Common Biochemical Property of Tumors Derived from Different Tissues[1]

Christopher Carruthers and Valentina Suntzeff

Division of Cancer Research,
Washington University School of Medicine,
St. Louis, Missouri

Previous investigations from this laboratory have demonstrated that polarographically reducible materials present in the epidermis of the mouse and man and in the liver and muscle of the mouse are structurally altered when these tissues become malignant (*1–3*). The reducible materials also absorb in the ultraviolet. Evidence of an alteration in the structure of the reducible material in the malignant transformation of epidermis to squamous-cell carcinoma was given by differences in the half-wave potentials and in the absorption characteristics in the ultraviolet of the material from epidermis as compared to that from the carcinoma (*3*). The data presented in this report further substantiate our previous results on a qualitative chemical change in carcinogenesis, and they also show that the tumors examined have a common biochemical property resulting from this alteration.

Methods for the extraction and partial purification of the reducible materials have been given (*3*). Briefly, the tissues were extracted with mixtures of alcohol and peroxide-free ethyl ether, and the total lipid thus obtained by evaporation of the solvents was re-extracted with dry ether, filtered, and the ether removed on a steam bath. Then the acetone soluble fraction of the total lipid, which contained the reducible material, was further fractionated by partitioning it between alcohol, acetone, and water saturated with petroleum ether against the latter saturated with alcohol, acetone, and water. The polarographically reducible material obtained in this manner represented 0.01–0.02% by weight of the fresh tissue. Then nonreducible compounds containing phosphorus were precipitated from the partially purified material in an alcohol-water mixture with calcium chloride (*3*). The latter was spun down at 0° C at 2,500 rpm, and the supernatant was dried at 56°–60° C in a vacuum oven. The dry residue was then treated with 4–6 ml ice-cold water, from which a colored nonreducible substance was separated by centrifugation at 0° C. If the reducible material at this stage was highly colored, much of the colored material could be removed by several extractions with 10-ml portions of peroxide-free ether. The reducible materials thus obtained are light-yellow in color, hygroscopic, soluble in water, alcohol, *N* butyl alcohol, *N* amyl alcohol, and only slightly soluble in nonpolar solvents. The materials are dialyzable through cellophane, stable to heat (steam bath), to storage at 0° to 4° C for a period of months and to oxygen. They appear to be nonprotein.

Some of the polarographic data obtained from the reducible materials are of interest since they may aid in determining whether the reduction is reversible, and in establishing the number of electrons involved in their reduction (*4*). Although the first wave of the double wave of the material from normal and hyperplastic epidermis (Table 1) appeared to be diffusion-controlled, since the diffusion current and the half-wave potentials were independent of the buffer used at constant pH, from pH 4.0 to 7.2, the relationship between i_d, the diffusion current, and h, the height of the mercury reservoir, was determined. For this experiment the purified material from hyperplastic epidermis was dissolved in 1.5 ml dioxane, 1.5 ml citrate buffer (0.1 *M*) of pH 3.16 (final pH, 4.2), and sufficient tetrabutylammonium iodide was added to make the solution 0.1 molar. The results are shown below:

h(Hg)	i_d(μA)	$i_d/h^{1/2}$
40.5	1.68	0.264
50.5	1.80	0.253
60.5	1.99	0.256
70.5	2.19	0.263
	Average	0.259

Since $i_d = Kh^{1/2}$, the reaction at the dropping mercury electrode is diffusion-controlled (*4*). Similar data were found for the material from the squamous-cell carcinoma.

The diffusion currents and the half-wave potentials were determined on the material from normal epidermis at 2°, 15°, and 25° C in citrate buffer, dioxane, and tetrabutylammonium iodide mixture of pH 6.4, and from liver in the same mixture buffered at pH 5.2 at 2°, 25°, and 40° C. From a plot of the diffusion current against the temperature, the slope of the straight line for the material from normal epidermis gave a temperature coefficient of 1.4%/degree; that from liver was 3.0%/degree. These coefficients are of the same order of magnitude as that of normal diffusion currents (*4*). Furthermore, the half-wave potentials were independent of the temperature, which may indicate a reversible reaction at the dropping mercury electrode (*4*).

In another set of experiments the materials from normal epidermis, squamous-cell carcinoma, liver, hepatoma, and rhabdomyosarcoma were polarographed as previously described (*2*) and $\log i/(i_d - i)$ was plotted against E_{dc}, the potential at the dropping mercury electrode (*4*). The reciprocal of the slope of the straight lines from the materials from these tissues

[1] This investigation was aided by grants from the Charles F. Kettering Foundation and the American Cancer Society.

TABLE 1

POLAROGRAPHIC AND ULTRAVIOLET ABSORPTION DATA ON SEVERAL TUMORS AND THEIR NORMAL HOMOLOGOUS TISSUES

Tissue	Half-wave potentials, $E_{1/2}$ (v) *	Diffusion current, i_d/mg material (μa)	Absorption maximum (mμ)	Extinction coefficient (ε)	$\frac{\varepsilon}{i_d}$
Normal epidermis	−1.40	0.86	282	7.90	9.24
Hyperplastic epidermis	1.39	.57	282	5.73	10.05
Muscle	1.28	.57	260 (Below pH 4.0)	3.32	5.84
Liver	1.28	.50	260	2.94	5.88
Squamous-cell carcinoma	1.28	.33	260	6.43	19.5
Rhabdomyosarcoma	1.28	.23	260	5.49	21.0
Hepatoma	−1.28	0.13	260	2.38	21.8

* Versus the saturated calomel electrode.

at pH 6.5–6.82 was 0.050 v–0.072 v, with an average of 0.058 v, which is in good agreement with the theoretical value of 0.059 v for a one-electron transfer. The half-wave potentials of the materials from the tissues measured from the plot of log $i/(i_d - i)$ against E_{de} were in excellent agreement with those determined experimentally.

A summation of the data on the reducible materials in some normal tissues and in the tumors derived from them is given in Table 1. The half-wave potentials, characteristic constants for any polarographically reducible substance or for a group of compounds under controlled conditions of electrolysis, in volts versus the saturated calomel electrode, were those obtained in buffered solutions at pH 4.13–4.20. The half-wave potential of the reducible material from normal and hyperplastic (methylcholanthrene-treated) epidermis is about 100 mv more negative than that of the substances from muscle, liver, or from the tumors. The half-wave potentials of the reducible materials vary directly with pH up to pH 7.0 in such a manner that this difference is maintained (*3*); hence only the values at pH 4.13–4.20 are shown. The polarographic waves disappear above pH 8.0 for the reducible materials for all the tissues examined.

In the third column of Table 1 is shown the amount of reducible material, expressed as diffusion current, a quantitative measure of the amount of the materials present, in μa/mg reducible material. (The amount of reducible material was determined on an aliquot of the total sample in vol of 2 ml.) In all cases the tumors contain much less than the tissue of origin. The materials also absorb in the ultraviolet (col. 4); the material from normal and hyperplastic epidermis absorbs maximally at 282 mμ, and the absorption is pH-dependent (*3*); that from muscle absorbs maximally at 260 mμ only when the pH is below 4.0, and that from liver absorbs maximally at 260 mμ from pH 1.5 to 12.0. The material from the tumors absorbs maximally at 260 mμ, and the absorption like that of liver is pH-independent. The absorption maxima of the above samples were measured in alcohol at approximately pH 5–6 (unbuffered) except for that from muscle, which was measured at pH 1.5 in a buffered solution. The extinction coefficients of the materials from the normal tissues and from their respective tumors are not too different except for that from the rhabdomyosarcoma, which is nearly twice that of muscle.

The ratio, extinction coefficient ε: diffusion current i_d, for the material from the tumors is nearly the same value of about 20 : 21, which is much greater than that of the materials from the tissues of origin. The increase in this ratio in the tumors is due to a reduction of the diffusion current, and also to an increase in the value of ε in the muscle tumor. Therefore, the materials in the three normal tissues are different, as indicated by their polarographic behavior or by their absorption characteristics in the ultraviolet, yet the tumors have the common property of a low diffusion current and a constant ratio of ε/i_d. The results presented in Table 1 are the averages of many determinations.

The pronounced difference in polarographic behavior and absorption characteristics in the ultraviolet of the material from epidermis as compared to that from the squamous-cell carcinoma greatly facilitated the discovery of the qualitative chemical change in carcinogenesis. Hence the value of epidermis for the chemical study of carcinogenesis, conceived some years ago by Cowdry (*5*), as the tissue of choice, becomes more apparent.

The reducible materials used in these experiments were not pure. However, the data indicated that polarographic reducibility and absorption in the ultraviolet were properties common to each of the materials present in muscle, liver, and epidermis. Proof of this correlation was necessary because in the malignant transformation of epidermis to squamous-cell carcinoma there is a simultaneous alteration in both polarographic behavior and in absorption characteristics in the ultraviolet of the material which might conceivably result from the alteration of two different compounds. In the transformation of muscle to rhabdomyosarcoma there appears to be only an alteration in the property of absorption in the ultraviolet, or conceivably a change in only one compound. Finally, in the liver-hepatoma system, there appears to be no qualitative chemical change in either property. In other words, if two compounds are involved in the transformation of these normal tissues to malignancies, both are altered in epidermis when it becomes malignant, one in the transformation of muscle to rhabdomyosarcoma, and none in the formation of hepatoma from liver.

Proof that reducibility and absorption in the ultraviolet were common to each of the materials in the

normal tissues was achieved by the countercurrent distribution method of Craig *et al.* (*6*). For this purpose about 10–20 mg of the partially purified reducible materials were dissolved in 10 ml water saturated with *N* butyl alcohol to which was added in a separatory funnel (glass-stoppered) 10 ml butyl alcohol saturated with water. Countercurrent distribution was then carried out using 9 separatory funnels. Equilibrium of the reducible materials in both phases was obtained by inverting the funnels 40 times. Then the water and butyl alcohol layers were collected separately from funnels 1 to 8 and from the top layer from funnel 0 into 50-cc Erlenmeyer flasks. The samples were dried in a vacuum oven at 55°–60° C. The dry material in each flask was then dissolved in 95% alcohol, and an aliquot was used for the determination of the optical density in a Beckman spectrophotometer at the wavelengths given in Table 1. Suitable aliquots from the alcoholic solutions were then dried *in vacuo* at 55°–60° C for polarographic analysis as previously described (*1*).

Since the reducible materials will be distributed in the two phases according to their distribution coefficients, if polarographic reducibility and absorption in the ultraviolet are properties common to one compound, both properties should have the same distribution pattern. This is exactly what we find for the reducible material from epidermis, the distribution pattern of which is shown in Fig. 1. The sum of the optical density of both layers, measured at 282 mμ, and the sum of the diffusion current of both phases are plotted against the number of separatory funnels. Maximum concentration of the reducible material occurred in funnel 6, with a good correlation between optical density and diffusion current in funnels 4–8. This correlation is perfect in all funnels in the water layer, but not in funnels 1–3 in the butyl alcohol phase. This lack of correlation in the latter solvent is due to the presence of a substance which is very soluble in butyl alcohol and which absorbs nonspecifically between 230 mμ and 260 mμ. Some of this material is also present in the water layer in funnels 1–3 and thereby prevents a good correlation in these funnels. The absorption in the ultraviolet was measured only at 282 mμ, since the shape of the absorption curves in both solvents in funnels 3–8 was about the same. The solubility of the reducible material from epidermis was greater in the water layer than in the butyl alcohol layer. Since the properties of polarographic behavior and absorption in the ultraviolet are distributed in a similar fashion in each layer, and are directly proportional to the solubility of the material in each phase, these properties must be common to one compound.

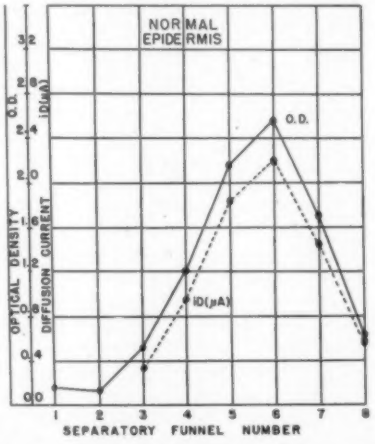

FIG. 1. Countercurrent distribution pattern of reducible substance from epidermis.

The reducible material from mouse liver was subjected to countercurrent distribution in the same manner as for epidermis. The material was concentrated maximally in funnels 3 and 4 with a good correlation between optical density and diffusion current in funnels 2–5 in each solvent. Some liver samples have shown a slight dissociation of the portion of the reducible material absorbing at 260 mμ in water phase, but never in the butyl alcohol phase. The material was slightly more soluble in the butyl alcohol layer, with respect to both measurements. The reducible material from muscle had a distribution pattern similar to that of liver with a good correlation between optical density and diffusion current in funnels 2–5 in each solvent. The solubility of the reducible material was slightly greater in the butyl alcohol phase. Furthermore, the material from muscle did not absorb at 260 mμ unless the pH was below 4.0. Since the properties of polarographic behavior and absorption in the ultraviolet are distributed in a similar fashion in each layer, and in proportion to the solubility of the material, these properties must be common to the materials from liver and muscle.

The absorption curves of the materials from muscle and liver in both solvents in funnels 2 to 5 were quite similar, so the optical density was measured only at 260 mμ. This was also the case for the materials for the tumors which absorb maximally at 260 mμ, and there was no essential change in the shape of the absorption curves at various pH levels. Furthermore, with the exception of the material from epidermis, the absorption maximum is pH-independent, muscle not absorbing above pH 4.0.

The material from the tumors was subjected to countercurrent distribution in the same manner as for the normal tissues, and the results for the material from the squamous-cell carcinoma are shown in Fig. 2. It is now apparent that the property of reducibility in both layers has concentrated in funnels 3 and 4, whereas that portion of the material absorbing at 260 mμ is concentrated maximally in funnel 6. Analyses of each phase showed that the distribution and solubility of the reducible portion of the material in both phases was about the same, whereas that portion of the material absorbing at 260 mμ was nearly

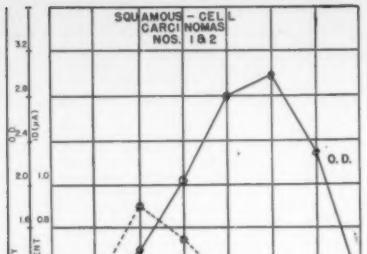

FIG. 2. Countercurrent distribution pattern of reducible substance from the squamous-cell carcinoma.

twice as soluble in the water layer as in the butyl alcohol layer. The material from the rhabdomyosarcoma and hepatoma showed about the same distribution and solubility in the two solvents as that from the squamous-cell carcinoma. These data demonstrate that the reducible material in the tumors has been cleaved, resulting in a common property of the tumors examined.

Since the fraction of the molecule absorbing at 260 mμ was about twice as soluble in water as in butyl alcohol, further evidence of cleavage was obtained by partitioning the materials between 5 ml *n*-amyl alcohol and 5 ml water. The separatory funnel containing the solvents was inverted 50 times to insure a good distribution. After complete separation of the phases, analyses of each were carried out as before. The results of this experiment are shown in Table 2.

From the data in Table 2 it is apparent that the reducible material of epidermis has a much greater solubility in water than in amyl alcohol, with a good correlation between optical density and diffusion current. The materials from liver and muscle have about equal solubility in both phases with respect to these properties. On the other hand, the cleaved portion of the material of the tumors, including a connective tissue sarcoma, absorbing at 260 mμ has about twice the solubility in the water phase as in the amyl alcohol phase, whereas the solubility of the reducible portion of the material, the amount of which is greatly reduced from that of the tissue of origin, is only slightly greater in the water layer. Hence these data on the substance from the tumors confirm those obtained by countercurrent distribution.

TABLE 2

DISTRIBUTION OF THE REDUCIBLE SUBSTANCES IN AMYL ALCOHOL AND WATER

Tissue	i_d water / i_d amyl alcohol	OD water / OD amyl alcohol
Epidermis	4.65	4.86
Liver	1.11	1.29
Muscle*	1.16	1.04
Squamous-cell carcinoma	1.09	1.91
Hepatoma	1.29	1.67
Rhabdomyosarcoma	1.18	2.26
Sarcoma,† connective tissue	1.19	2.07

* At 0° C.
† Originally induced by methylcholanthrene.

Proof that the reducible portion of the material in the tumors has resulted from cleavage of the parent material present in the tissue of origin is given by the following common polarographic properties of both: (1) The half-wave potentials, except epidermis, and their dependency on pH are the same; (2) the polarographic waves disappear above pH 8.0; (3) iodide ion or some other property of tetrabutylammonium iodide, the supporting electrolyte, is necessary for their reduction; (4) a one-electron transfer is involved in their reduction.

Since the properties of polarographic reducibility listed above are the same for the materials as found in the normal tissues and for the cleaved material in the tumors, the reducible portion of the material and that part absorbing at 260 mμ must have come from

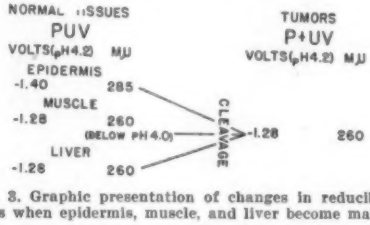

FIG. 3. Graphic presentation of changes in reducible substances when epidermis, muscle, and liver become malignant.

the parent materials. Further evidence that this is true is given in the following data for both the parent materials in the normal tissues, except epidermis, and for the cleaved material in the tumors which absorb at 260 mμ: (1) The absorption curves have the same general shape with a maximum at 260 mμ; (2) the maximum is not affected by changes in pH (substance from muscle does not absorb above pH 4); (3) there is an increase in the extinction coefficient of about 10% at pH 1.3–1.5; (4) there is a similar ratio of maximum at 260 mμ to minimum at 240 mμ; (5) there is a decrease in this ratio (4) at pH 12.0. A more rigorous proof of this correlation will come from infrared spectroscopy and other techniques.

The manner by which the tumors cleave the reducible materials as present in the tissues of origin has not yet been investigated. One might suspect that cleavage has resulted enzymatically, in which case the enzymes responsible for splitting the reducible materials would be either absent or inhibited in the normal tissues. Since the tumors studied are derived from ectoderm (epidermis), endoderm (liver), and mesoderm (muscle), a survey of tumors of other organs

seems advisable to ascertain whether the change in the reducible materials is a general phenomenon.

The evidence presented shows that polarographically reducible materials that are characteristic for epidermis, muscle, and liver are cleaved when these tissues become malignant. A graphic presentation of these changes is shown in Fig. 3. Since the materials are reducible polarographically (P), and since they absorb in the ultraviolet (UV), let PUV stand for the materials as present in the three normal tissues, for both properties are common to each material. Differences in the polarographic and absorption characteristics (or PUV) in the materials from epidermis, muscle, and liver are given in the half-wave potentials and in the absorption maxima. In spite of the fact that these differences exist in the normal tissues, in tumors derived from the latter, cleavage of the parent material, PUV by the tumors into $P + UV$, or two distinct components, has occurred. The half-wave potentials and the absorption maximum of the cleaved products in the tumors examined are the same. In other words, cleavage has resulted in a common biochemical property of these tumors. The nature of the alteration of the reducible materials, particularly in epidermis and also in muscle, suggests that they are altered antecedent to, or concomitant with, cleavage in malignancy. If the alteration of these materials is directly associated with the process of malignancy, a rational approach to the chemotherapy of cancer might follow.

References

1. Carruthers, C., and Suntzeff, V. *Science*, **108**, 450 (1948).
2. ———. *Cancer Research*, **9**, 210 (1949).
3. *Ibid.*, **10**, 339 (1950).
4. Kolthoff, I. M., and Lingane, J. J. *Polarography*. New York : Interscience (1946).
5. Cowdry, E. V. *Biol. Symposia*, **10**, 131 (1943).
6. Craig, L. C., *et al. J. Biol. Chem.*, **161**, 321 (1945).

Comments and Communications

Scientific Manpower

An abstract of a report entitled "Why Not a Department of Science and Technology within the Department of Defense?" appeared in the January 1951 issue of the *Chemical Bulletin*. This report outlined a plan for the efficient integration of our military research efforts into a single department concomitant with effective utilization of scientific manpower, facilities, and funds. It is of interest that the Canadian Ministry of Defence has been so organized since 1946.

We believe that the plan is consistent with the resolution on scientific manpower passed by the Council of the American Association for the Advancement of Science December 29, 1950.

As individuals at present actively engaged in research and development in the fields of chemistry, engineering, mathematics, and microbiology who also saw active military service in World War II, we urge that this proposal be given serious consideration.

The following excerpts have been taken from the above-mentioned report:

National safety is dependent on the successful operation of the Department of Defense, which, in turn, is determined by the efficiency with which our natural advantages are employed and detrimental factors eliminated. In terms of manpower, this means that our resources of talent and training must be recognized and made use of under conditions most conducive to the productiveness of the men concerned. Timely utilization of the work potential of men expert in their chosen professions (a factor of considerable importance to morale) may do much to offset any numerical advantage in manpower possessed by potential enemies.

Such reasoning is particularly applicable in the accomplishment of scientific and technical work, which is increasingly important to military operations. Conservation of critical natural resources can be augmented by early research, leading to the more efficient employment of present supplies, and to the development of adequate substitutes. Weapon superiority can only be achieved by constant research and development both in design and in tactical employment. With few exceptions, improvements in the design of weapons and equipment have come in the period preceding war.

It follows that activities and direction in the fields of science and technology are of direct concern to the national defense in times of peace as well as in wartime.

. . . a Department of Science and Technology should be established within the Department of Defense. Let this Department of Science and Technology be headed by a Secretary equal in status to the Secretaries of the Army, the Navy and the Air Force.

All of the research activities and installations now under the jurisdiction of the military services should be assigned to the Department of Science and Technology, making provision for the closest liaison between the Department of Science and Technology and the other three branches of the Department of Defense. The latter would then be free to concentrate on their primary objectives of combat, procurement and supply, assuming only the responsibility for the final development and field testing. . . .

. . . The establishment of such a department would offer the following advantages:

1. Integration of military research on a national level, avoiding duplication of effort and expenditure.
2. Assurance of technically competent superiors to direct scientists, resulting in greater efficiency and greater attraction for competent scientists to peacetime association with the Department of Defense.
3. Operation of a Scientific Personnel Selection Board which would, in an emergency function in the—
   a. Selection of personnel for military research and development.

b. Assignment of technical personnel to the Army, Navy and Air Force.

c. Selection of promising youths for educational deferment and training as scientists.

d. Adjudication of the relative requirements for scientists by the Armed Forces, industry, educational institutions and government bureaus.

4. Personnel promotional policies and physical standards can be designed to fit the requirements of scientific rather than military pursuits. . . .

. . .

6. Scientists in the higher echelons of the department would gain the experience in large-scale administrative procedure which would eliminate the need for hasty expedients such as were resorted to in World War II.

7. A scientific intelligence and analysis group as advocated by Bush would function in time of peace as well as war.

8. Administration of the Department by scientists would assure greater continuity of program, and minimization of financial fluctuations. Thus private contractors would be less reluctant to engage in such programs.

9. The plan offers maximum economy of money, manpower and facilities for scientific military requirements.

The plan is consistent with the policy of unification of the Armed Forces. It also is consistent with the recommendations of the Hoover Committee, in that certain independent executive bureaus, such as the Munitions Board, National Security Resources Board, National Advisory Committee on Aeronautics, etc., could and should be integrated into the new Department.

The Department of Science and Technology, as envisioned in this report, would provide the necessary position of the scientist in the over-all planning for the defense of the United States in the capacity stressed so emphatically by Vannevar Bush as an absolute requisite for national safety.

E. L. Hess
S. E. Owen
H. V. Parsley
W. T. Scott
H. D. Slade

Committee on Utilization of Scientists by the Department of Defense
Chicago, Illinois

The Reservist Problem

I wish to report another facet of the government's mobilization policy which damages the defense effort. This is the indiscriminate recall of reservists from civilian life. With no excuse but the overriding needs of the military, the Services have grabbed men with no consideration of their civilian experience and education. If they had no choice at the time, they have made no attempt to remedy the situation where malassignments were made.

The flaw in the reserve program, if it could be called one, lay in the premise that a reservist was a man who should come on duty at a moment's notice and carry on the same duties he had performed in a previous war. Screening and reclassification of reservists were talked about, but they never occurred, and one doubts whether they would be done properly. So, when the Korean war broke out, the reservist found himself to be just another available body. As reservists have not been hermetically sealed in cans since 1945, one finds examples such as these:

1) A Ph.D. in dairy chemistry is serving again as a junior officer, doing administrative work.

2) A geologist who applied for release documented the oil pools he had discovered and the wells he had brought in to prove he was more valuable as a civilian. He was told there was "no critical shortage in his field."

3) A civil engineer with prestressed design experience was put to work computing cubages of buildings, that being the most use the Corps of Engineers could make of his technical abilities.

4) A candidate for a Ph.D. in mathematics, lacking a few hours of his degree, now counsels men about night school courses.

5) A chemical engineer with experience in materials testing now works at supply matters, while the Air Force combs the country for materials engineers. They are probably being hired away from industries which need them more than the Air Force.

The Congress does not seem to realize the damage that has already been done by giving the military a free hand in grabbing reservist manpower. It is the equivalent of giving a Swiss music box to a gorilla.

A recent example of the danger we are in occurred at an electronics plant being toured by Air Force officers. They found a standard piece of equipment that met requirements, but it had to be modified to another range of frequencies. The company would have been glad to do this, *if* the engineers had had time. They did not because they were understaffed and very busy with other defense contracts. It is ironic that the Air Force is taking all the electronics people it can get, and is assigning them to semitechnical administrative or operational work.

The Services' attitude toward scientific manpower seems to be based on this outlook: (1) Their manpower needs come before all others, even if their policy will eventually damage them from a materiel standpoint. (2) They prefer people with technical or scientific backgrounds, even if they have no appropriate duties for them to perform. (3) They look upon the reservists and draftable students with a proprietary interest, as if the economy or the general welfare of the nation had no claims on their services.

Such a policy will be disastrous if they mean to keep us in a continuous state of partial mobilization. Their present misuse of scientific manpower can be justified only if we are on the eve of a short all-out war. How do they expect to multiply the effectiveness of each soldier by superior equipment, when many of the people who can bring this about are recalled and kept in uniform? The military should be required to answer these questions, and their manpower needs should be carefully screened by civilians who have a better over-all outlook.

From the present attitude, coupled with the damage that was done in the last war, we can be reasonably certain that they would not hesitate to put us on the road to scientific suicide.

RESERVIST
(*Name withheld by request*)

Microfilm Publication

I AM very much concerned about the petition submitted by the two committees on zoological nomenclature to the International Commission on Zoological Nomenclature reported in SCIENCE (113, 466 [1951]).

I think these committees have taken an extremely narrow point of view on a subject of great importance to both zoological and botanical nomenclature. The acknowledged shortage of publication space and cost of letterpress, lithoprint, etc., types of publication alone make it imperative that *every type* of publication that is readily available to the public be considered as a legitimate place of "publication" for taxonomic entities.

The paper cited cannot be used as an argument for their petition, for it is only an argument against the waste of money on republishing a paper already effectively published and available to anyone desiring it.

LEROY H. HARVEY
Department of Botany, Montana State University

Nondiffusibility of Alkaline Phosphatase in Fixed Tissue

DR. NOVIKOFF'S intensive examination of the histochemical tests for alkaline phosphatase (*Science*, 113, 320 [1951]) still leaves unanswered the question of whether the enzyme itself diffuses. That the enzyme does not diffuse during incubation of sections in aqueous medium at pH 9.4 can be shown by a simple test that, to my knowledge, has not appeared in the literature. In this laboratory we have made the test on sets of five slides of mouse duodenum, which are treated as follows:

1) A slide is incubated in standard Gomori medium at 38° for 5 sec. Appropriate further treatment then reveals a dense black precipitate in the striated border, but no sign of activity anywhere else.

2) Another slide is incubated in the medium for 30 min. After conversion of the precipitated calcium phosphate to cobalt sulfide, the entire section appears blackened, with a gradient of darkness extending away from the striated border through the epithelial cells, the intravilline stroma, and the mucosa and musculature. The Golgi bodies are darker than the rest of the cytoplasm. The picture certainly suggests diffusion from the border into inactive material.

3) Three other slides are incubated in barbital buffer (pH 9.4) at 38° for 30 min, and are then placed in buffer-substrate medium for 5, 15, and 30 sec. The pictures obtained after this treatment are the same as in case 1, with the precipitate being strictly limited to the striated border. There was no evidence of diffusion beyond the border, nor was there any apparent loss of enzymatic activity such as Yokoyama, Stowell, and Mathews (*Anat. Record*, 109, 139 [1951]) observed under somewhat similar conditions.

Of course these results do not bear on the possibility that alkaline phosphatase diffuses during fixation. They do, however, show that highly concentrated phosphatase does not alter its position in fixed and mounted sections kept in fluid medium at incubating temperature for as long as ½ hr. This finding is in agreement with Dr. Novikoff's demonstrations that it is possible for calcium phosphate to diffuse and be absorbed at false localizations in mounted sections.

FLORENCE MOOG
Department of Zoology, Washington University

Sui Generis

I READ with interest J. R. Pierce's article on "Science and Literature" in your issue of April 20, but I would like to point out one omission in it. He spoke of a book by Heinlein, tracing the imaginary future of man through many periods but omitted to mention what, in my opinion, is by far the best book on this subject, namely, Olaf Stapledon's *Last and First Men*. This pursued the subject in a most illuminating way, on the assumption that with the vast amount of time still ahead of the human species, it might well produce a succession of totally different types. Stapledon's picture of the society in which all the thinking was done by specialized individuals whose brains were cultured out to a gigantic size on some sort of trellis, is unforgettable!

JULIAN S. HUXLEY
London, England

A Note to the Department of Internal Revenue

THE appearance of the comments on "Scholars and the Root of All Evil" in SCIENCE (113, 330 [1951]) on March 23, at a time when scholars along with the rest of the tax-paying public were emerging from the annual struggle with income tax returns, started a trail of thought that poses another point for public attention. In reading the comments in SCIENCE we were confronted with Bauer's formula for deriving an approximately just and fair income for the scientist or scholar who has invested many years of his youth, many dollars of a then nonexistent income, many IQ points of mental capacity, and unbounded personal energy and zeal in preparing his mental equipment for lifetime service.

In making out the federal income tax return we noted the possible channels open to the businessman who also has invested money in ideas but who, on the other hand, has transmitted his investment into material things: buildings, equipment, inventories, etc., against which, in time, the government will allow a proportionate mark-off under a heading on page 2 called "depreciation." By putting the two investments in juxtaposition, the reader discovers that for the learned man, the one who has salted away his money and time and effort and ability in his "brains"—in

mental rather than material equipment—unlike the businessman, there is allowed no deduction for "depreciation."

Yet consider how definitely for some—though less perceptibly but still as surely for others—often how suddenly, the economic returns on the scholar's mental equipment terminate when his professional life becomes "depreciated" on retirement! Directly or indirectly, after a lifetime of study, of labor, of devotion to work, of repeated expenditures for scholarly "education"—the tools of the scholar's trade—the economic returns stop! Yet there is no allowance for him on page 2 under "depreciation"! Match this with the consideration given the nonacademic businessman just around the corner or down the street!

I am therefore adding this note to the current comments: Let mathematically inclined men like Dr. Bauer continue to work out a formula for a fair return on a man's professional and scientific investment, but let them also work out another formula, one that will enable the scholar's big educational investment to get recognition on the federal and state income tax returns in terms of deductions from the total capital outlay!

And then have the AAAS present the formula, with the full backing of all American scientists, to the Department of Internal Revenue, or to the legislators who make the laws controlling the workings of the Department of Internal Revenue, and have some effort made to get for scholars a break similar to that given businessmen.

GLADYS C. SCHWESINGER
California Youth Authority, Ventura

Observations on Purine Metabolism

IT IS possible that investigators in the past have placed undue emphasis on the integrity of the purine ring once it is formed. This consideration applies both to the utilization of exogenous purines and to the conversion of adenine and 2,6-diaminopurine to guanine. The evidence that has been put forth to support the retention of the intact ring system (*1, 2*) shows, on closer inspection, that ring opening may have taken place. Indeed in one case the latter hypothesis is supported by the very evidence cited to disprove ring opening (*2*).

In the first case (*1*) the guanine isolated from the rat viscera after feeding 1,3-N^{15}-adenine was degraded to xanthine and guanidine, and it was shown that all the isotope was retained in the 1- and 3-positions. These results do not rule out the possibility of ring opening between the 1- and 3-positions, or in the imidazole ring. In the second case cited (*2*), 2,6-diaminopurine was fed to rats in two different experiments and the guanine isolated from the rat nucleic acids. In the first diaminopurine experiment the purine was labeled in the 1- and 3-positions and in the 2-amino group with N^{15}, and of the guanine isolated 4.0% had been synthesized from dietary 2,6-diaminopurine. (At this point a degradation of the isolated guanine to xanthine would have been of interest.) In the second diaminopurine experiment, the purine was labeled with C^{13} in the 2-position, and the guanine isolated contained only 1.5% of isotopically labeled molecules (based on the administered diaminopurine as 100). No explanation for this difference was given, but the C^{13}-guanine was degraded to guanidine, which was shown to contain 85% of the isotope present in the guanine. This result was cited to show extensive retention of the ring system. Actually it shows, first, that the 2-carbon of 2,6-diaminopurine is biologically labile, and the pyrimidine ring must therefore open, and, second, that an appreciable amount of isotope seems to be reincorporated. This reincorporation may well be at the 8-position, and if this is the case the imidazole ring as well must be opened and recyclized during the interconversions, possibly during riboside formation. Feeding experiments with 8-labeled adenine are under way to test the possibility of the imidazole ring being opened and recyclized during incorporation of the purine.

There is a great deal of other scattered evidence in the literature which points to the possibility of a complex path for the incorporation of exogenous purines, as well as for the *in vivo* interconversions among the purines. The limited incorporation of guanine (*1, 3*), hypoxanthine (*4*), xanthine (*4*), and uric acid (*5*) into mammalian nucleic acids is illustrative of the poor utilization of preformed purines. Further, the participation of a ring-opened intermediate in microbial metabolism, as well as in mammalian, is indicated by the fact that the inhibition of growth by antifolics is reversed only by large amounts of preformed purines, if at all, even though folie acid is certain to be importantly involved in purine metabolism (*6, 7*).

These matters are of importance since the design of suitable purine antagonists as tumor-inhibiting agents has been the goal of a number of investigators (*8–10*). If our hypothesis is correct, the synthesis of purine analogs containing intact rings may be a less fruitful line of research than the preparation of suitable open chain or monocyclic compounds, perhaps conjugated with formylfolic acid (as a Schiff base), with ribose, or with both.

MAXWELL GORDON
Organic Chemistry Department
Imperial College of Science and Technology
London

References

1. BROWN, G. B., *et al. J. Biol. Chem.*, **172**, 474 (1948).
2. BENDICH, A., FURST, S. S., and BROWN, G. B. *Ibid.*, **185**, 426 (1950).
3. PLENTL, A. A., and SCHOENHEIMER, R. *Ibid.*, **153**, 203 (1944).
4. GETLER, H., *et al. Ibid.*, **178**, 259 (1949).
5. BROWN, G. B., ROLL, P. M., and CAVALIERI, L. F. *Ibid.*, **171**, 835 (1947).
6. GORDON, M., *et al. J. Am. Chem. Soc.*, **70**, 878 (1948).
7. TSCHESCHE, R. *Angew. chem.*, **62**, 153 (1950).
8. HITCHINGS, G. H., *et al. J. Biol. Chem.*, **174**, 765 (1948); BURCHENAL, J. H., *et al. Cancer*, **2**, 119 (1949).
9. ROBLIN, R. O., *et al. J. Am. Chem. Soc.*, **67**, 290 (1945); KIDDER, G. W., *et al. Science*, **109**, 511 (1949).
10. GORDON, M., SIRI, J. B., and CAMPBELL, J. G. *Ibid.*, **113**, 61 (1951).

Association Affairs

Gordon Research Conference
June 18–August 31, 1951

As announced in SCIENCE April 27 (113, 499 [1951]), the Gordon Cancer Conference will be held at Colby Junior College, New London, New Hampshire, August 27–31. Details of the program follow.

CANCER

August 27: A. Cantarow, "Production of Tumors with Fractions of Mammalian Neoplasms;" Hans Lettré, "Behavior of Components of Tumor Cells in Transplantation;" F. Duran-Reynals, "On Tumor Viruses and the Virus Theory of Cancer: Carcinogenic Potentialities of Some Pox Viruses."

Round Table: "Fowl Tumors." *Moderator*: Jacob Furth. *Invited participants*: Joseph W. Beard, W. Ray Bryan, Ben R. Burmester, W. H. Eyestone, C. O. Prickett, F. Duran-Reynals, George R. Sharpless, E. W. Shrigley, Nelson F. Waters.

August 28: G. Burroughs Mider, "Effects of Tumors on Host Metabolism;" L. W. Law, "Transformations in Tumor Cells and the Problem of Chemotherapy;" Alfred Gellhorn, "Present Status of Cancer Chemotherapy."

Round Table: "Screening Techniques for Cancer Chemotherapy." *Moderator*: Sidney Farber. *Invited participants*: E. Boyland, Louis H. Goodson, Alex. Haddow, George H. Hitchings, J. Leiter, Hans Lettré, Ira T. Nathanson, C. Chester Stock, Herbert C. Stoerck, J. H. Williams.

August 29: Arnold M. Seligman, "Development of Histochemical Methods for Intracellular Enzymes;" Albert J. Dalton, "Electron Microscopy of Cytoplasmic Components of Mammalian Cells;" Sidney Weinhouse, "Oxidative Mechanisms of Tumors."

Round Table: "Environmental Cancer." *Moderator*: Paul E. Steiner. *Invited participants*: Austin M. Brues, Alexander G. Gilliam, Alex. Haddow, Wm. C. Hueper, Morton L. Levin, Willard Machle, A. A. Nelson, John Rehner, Jr., Robert Shrek, Wm. E. Smith.

August 30: Tomizo Yoshida, "Some Cancer Problems Studied with Yoshida Sarcoma;" Mrs. H. Lettré, "Variations in Cell Shape under Influence of Environment in Tissue Culture;" Frank L. Rose, "Carcinogens and Cancer Therapy."

Round Table: "Mechanisms in Tumor Genesis." *Moderator*: Albert Tannenbaum. *Invited participants*: E. Boyland, C. Carruthers, W. U. Gardner, J. L. Hartwell, Charles Heidelberger, Elizabeth C. Miller, P. Shubik, Harold L. Stewart.

August 31: Richard J. Winzler, "Carbohydrate-containing Protein in Human Blood Serum;" M. R. Shetlar, "Polysaccharide Levels in Human Blood Serum;" I. A. Parfentjev, "Immunochemical Analysis of Human Blood Serum for Labelling of Malignancy;" Harry S. Penn, "Flocculation Reactions in Human Blood Serum."

Academies

AAAS research grants have recently been awarded as follows:

Illinois Academy of Science

C. W. Bennett, Chemistry Department, Western Illinois State College, for research on microanalyses of nitroderivatives of fluorene

Sister M. Joan, Chemistry Department, College of St. Francis, Joliet, for research on the improvement of taste of hothouse vegetables

James W. Mitchell, Shawneetown Community High School, for research on a tapeworm parasitic in the liver of the fresh-water perch

D. R. Smith, Chemistry Department, James Millikin University, Decatur, for research on the extension of the Leuckart reaction for the preparation of tertiary amines

Kansas Academy of Science

Roland S. Fischer, University of Kansas, for a preliminary classification of the North American Megachilidae based on the morphology of the male genitalia

Robert Gibbon, for research on the root and top growth of five native Kansas legumes from seed to end of first season, and an anatomical study of these plants

North Carolina Academy of Science

Maurice Whittinghill, University of North Carolina, for a genetic study of rheumatoid arthritis

Academy of Science of St. Louis

Carl Kisslinger, Institute of Technology, St. Louis University, for research on the difference in seismic velocities in calcium carbonate and magnesium limestone in place

Virginia Academy of Science

John Thornton Wood, Virginia Fisheries Laboratory, Gloucester Point, for field work in Virginia for the collection of herpetofauna

Wisconsin Academy of Sciences, Arts and Letters

Lester W. J. Seifert, Department of German, University of Wisconsin, for a survey of the German spoken in Wisconsin

---

The second **International Congress of Biochemistry** will be held in Paris July 21–27, 1952. Four general lectures and seven symposia are planned. Biochemical societies and departments, and institutes of biochemistry, may expect to receive the provisional program, forms for admission, and other information this summer. Authors should send titles of their papers before *March 1*, and summaries of less than 200 words before *April 1*, to the General Secretary, J. E. Courtois, 4, Ave. de l'Observatoire, Paris VI°.

At the triennial meeting of **Iota Sigma Pi**, women's national honor society in chemistry, Florence R. Sabin was awarded a certificate of National Honorary Membership, and Charlotte Roderuck of Iowa College was the recipient of the first research award. Gladys A. Emerson was elected president, and Margaret G. Morehouse vice president. Other officers elected were: Anna L. Hoffman, secretary; Anna Marie Duval, treasurer; and Elizabeth L. Knapp, editor. The 26th Iota Sigma Pi chapter was installed at Hunter College in June.

News and Notes

Scientists in the News

The Texas Gulf Sulfur Company has announced the election of **Walter H. Aldridge** as board chairman, **Fred M. Nelson,** president, and **Thomas S. Lamont** as chairman of a new executive committee. Mr. Aldridge has been president of the company throughout its 32 years. Mr. Nelson, who succeeds him as president, since joining the company in 1927 has been in charge of exploration and production. As executive assistant from 1945 to 1950 he was in charge of sour gas investigations. Since last year Mr. Nelson has been executive vice president of a company subsidiary in Mexico.

The Department of State has announced foreign missions for **Reginald M. Atwater, Joseph F. Volker,** and **Philip Bard** under its Exchange of Persons Program. Dr. Atwater, executive secretary of the American Public Health Association, left in June for a two-month lecture tour in Mexico, Panama, and several South American countries. Dr. Volker, dental dean at the University of Alabama, has gone to Thailand to spend four months as a consultant in dental techniques and to assist in a dental hygiene mass-education program. Dr. Bard, head of the Department of Physiology at Johns Hopkins, is delivering a series of lectures at the University of Chile this month.

Boris A. Bakhmeteff, authority on hydraulic engineering and water problems, and chairman of the AAAS Section on Engineering, has been named an honorary professor of civil engineering by the trustees of Columbia University. Apart from a distinguished career as an engineer and consultant on hydraulic problems, Dr. Bakhmeteff served as Russian Ambassador to the United States under the Kerensky government. Since 1931 he has been professor of civil engineering at Columbia. Dr. Bakhmeteff is the fourth person in Columbia's history to be honored by an appointment as honorary professor.*

Under the joint sponsorship of the Iowa State College Institute for Atomic Research and Division of Science, **R. M. Barrer,** of the Chemistry Department, Marischal College, Aberdeen, Scotland, will visit the institute September 15–22 and will deliver three lectures on researches on minerals. **G. Schwarzenbach,** of the University of Zurich, will deliver 12 lectures on solution complexes, beginning September 20. **John Lennard-Jones,** of the University Chemical Laboratory, Cambridge, Eng., will lecture on the theories of molecular structure from November 19 until December 1.

Hussein Kamel Selim Bey, dean of the Faculty of Commerce at Fouad University and member of the Egyptian delegation to the Fifth General Assembly of the United Nations, headed the Egyptian Town Hall Mission, which recently returned the friendly call of America's Town Meeting of the Air. Town Hall, Inc., of New York, managed the American tour of the Egyptian group.

John J. Bittner, director of the University of Minnesota's Division of Cancer Biology, has been named first winner of the Comfort Crookshank Award for Cancer Research, an honor presented through the Middlesex Hospital Medical School in London. Dr. Bittner will go to London in September to receive the award and at the same time will deliver a lecture on his research at the Middlesex school. The award, from funds donated by the late Bessie C. Crookshank, will be presented triennally to some scientist who has made valuable contributions to cancer research. Dr. Bittner, discoverer of the milk agent in mammary cancer in mice, is past president of the American Association for Cancer Research and winner of the 1950 medal of the American Cancer Society.

Sidney Doree Black has joined Horizons, Incorporated, as division supervisor in experimental physics.

Robert G. Bloch, professor of medicine, Section on Tuberculosis and Diseases of the Chest, University of Chicago, has resigned to accept the position of director of the Division of Diseases of the Chest at Montefiore Hospital, New York.

W. L. Burlison will retire on September 1 as head of the Department of Agronomy at the University of Illinois. He will be succeeded by **M. B. Russell,** professor of soil physics at Cornell.

Recent visitors at the Communicable Disease Center, USPHS, Atlanta, Ga., were **Joseba Kelmendi de Ustaran,** School of Hygiene, Santa Fé, Argentina; **Mario Sant' Ana,** State Department of Health, São Paulo; **Llewelyn F. Gunaratna,** Anti-Malaria Campaign, Colombo, Ceylon; **Anastasios Zairis,** Greek Ministry of Health, Salonika; **Ganga Prasad Chakravarti,** United Provinces Public Health Service, Pratapgarh, India; **M. L. Loganathan,** Mysore Public Health Service, Bangalore, India; **Leonard Jan Bruce-Chwatt,** Malaria Service, Medical Department, Lagos, Nigeria; **Consorcia Bautista,** Manila; and **A. Kohn,** Weizmann Institute of Science, Rehovoth.

Merriam A. Jones, specialist in agricultural chemistry, has left on a Point IV technical cooperation assignment in Guatemala. He will join a group of seven OFAR technicians already at work at the jointly operated agricultural station there. Dr. Jones joined the Department of Agriculture in 1939 and served for six years at the agricultural experiment station at Mayaguez, P. R. Since 1946 he has been a research chemist in the Bureau of Agricultural and Industrial Chemistry.

* News of Dr. Bakhmeteff's death on July 22 at his summer home in Brookfield, Conn., came as this issue of SCIENCE went to press.

Luiz Carlos U. Junqueira has been appointed professor and head of the Department of Histology and Embryology of São Paulo University Medical School. A laboratory for cell physiology is under organization in this department, aided by grants from the medical school and the Rockefeller Foundation.

Alfred Kahler, professor of economics in the graduate faculty of political and social science of the New School for Social Research, has been elected dean of the faculty. Dr. Kahler has been a member of the graduate faculty since 1934.

The Vitamin A Research Unit of the Bureau of Human Nutrition and Home Economics, under the leadership of **Elsa Orent Keiles,** nutrition chemist, has received the Distinguished Service Award presented by the U. S. Department of Agriculture. **Elizabeth C. Callison** had chief responsibility for the physiological aspects of the research. Miss Keiles has since been transferred to the Division of Research Grants and Fellowships, NIH.

Robert T. Lagemann, of Emory University, has accepted the chairmanship of the Department of Physics at Vanderbilt University. **F. G. Slack,** previously head of the department, has resigned and will make his home at Winter Park, Fla.

Victor Lorber, associate professor of biochemistry at Western Reserve University, has been appointed the first Career Investigator of the American Heart Association. He will receive a starting stipend of $12,000 to conduct research relating to disorders of the heart and blood vessels, plus $7,500 per year for technical assistance and supplies. The institution in which he will work will receive $1,000 annually for overhead. The AHA intends to continue the support during the productive life of the researcher, and hopes to finance other Career Investigators.

Giano Magri, of Ferrara, Italy, Morris Fishbein, Jr., fellow, has arrived in the U. S. to undertake studies of rheumatic heart disease in children. He will work under the direction of **Aldo A. Luisada,** program director of cardiology and assistant professor of medicine, Chicago Medical School, dividing his time between the school laboratory at Mount Sinai Hospital and La Rabida Sanitorium.

Ernst Mayr, of the American Museum of Natural History, has been made an honorary member of the Kebun Raya Indonesia (Botanic Gardens of Indonesia). Others given this honor at the same time were: **Eduard Handschin,** director, Naturhistorisches Museum, Basel; **Dirk Fok Van Slooten,** of Amsterdam; and **August Adriaan Pulle,** of Utrecht.

Alexander Murray, an Eastman Kodak research scientist, has been named the outstanding person in the graphic arts industry for 1951 by the Technical Association of the Graphic Arts Industry. He received the award for his contribution of a long series of improvements to the printing industry. The award cited particularly his work on tone reproduction and dot etching problems.

Edmund W. Oesterreich, who developed the "pole top" method of artificial respiration for the resuscitation of electric shock victims, has received a citation for distinguished service and a special commemorative bronze medal from the Edison Electric Institute. The Osterreich method, an adaptation of the Schaefer method, is credited with saving the lives of more than 100 linemen since its first use in 1931.

Egon Orowan has been appointed George Westinghouse professor of mechanical engineering at MIT, succeeding **William R. Hawthorne,** who has held the Westinghouse chair since 1948. Professor Hawthorne is resigning to accept the post of the Hopkinson and Imperial Chemical Industries professorship of applied thermodynamics at Cambridge University.

Armand J. Quick, professor of biochemistry, Marquette University School of Medicine, has left for South America to lecture and give demonstrations on the coagulation of blood and the hemorrhagic diseases. He was invited by the Society of Biology of Santiago, University of Chile, Foundation for the Study of Hemophilia, National Academy of Medicine, Buenos Aires, Argentina Society of Hematology and Hemotherapy, Medical School of Montevideo, and the Medical School of São Paulo.

Ernest W. Reid, president of the Corn Products Refining Company, has been chosen to receive the Chemical Industry Medal for 1951. The medal will be presented formally to Mr. Reid at a meeting following a dinner in his honor in the Waldorf-Astoria next November. The medal was established in 1933 and is awarded annually in recognition of conspicuous services to applied chemistry.

Emanuel B. Schoenbach has resigned as associate professor of preventive medicine, assistant professor of medicine, and physician at the Johns Hopkins Hospital as of August 31. He will become professor of medicine at the State University of New York College of Medicine, and director of the medical services at the Maimonides Hospital of Brooklyn.

William A. Scholes and **Harold N. Barr,** ceramic engineers of the Fairchild Engine and Airplane Corporation at Oak Ridge, have joined the ceramics and minerals department at Armour Research Foundation. Dr. Scholes will study the development of refractory container materials suitable for melting titanium and its alloys, and Mr. Barr will study pressing materials in heated molds.

Ellis L. Spray has been elected a vice president of the W. L. Maxson Corporation. Formerly a vice president of Westinghouse Electric Company, Mr. Spray will be in charge of the new Maxson plant in Old Forge, Pa.

Mary M. Thompson, director of the infirmary and

associate professor of nursing at the New Jersey College for Women for the past 31 years, has retired. A graduate of the St. Luke's Hospital Nursing School in New York, Miss Thompson served in Europe as a nurse in World War I. She came to the New Jersey College for Women as head of the infirmary in 1920.

Ralph M. Thompson, staff member at the Air Force School of Aviation Medicine, has been selected to direct Air Force activities at the Armed Forces Institute of Pathology in Washington, D. C. Col. Thompson has been head of the Department of Pathology at the aeromedical school for the past year. The Institute of Pathology was founded as the Army Medical Museum in 1862 by Surgeon General William A. Hammond, to study wounds and diseases, with the object of reducing mortality and suffering among soldiers. The Army Medical School was established at the Museum in 1893. After World War II the facilities for diagnosis and research were expanded so widely that its name was changed to the Institute of Pathology. Since 1949 it has been the central laboratory for all three branches of the armed forces. A new building to house the institute is now under construction on the grounds of the Army Medical Center in Washington, replacing the old structure next door to the Smithsonian Institution.

E. W. Titterton, of AERE, Harwell, Didcot, Berks., is now in Canberra to assume his duties as professor of physics in the Research School of Physical Sciences, the Australian National University.

B. Bynum Turner has been elected a vice president of Ethyl Corporation in charge of a new department which will consolidate all research, engineering, product development, and construction activities. **Graham Edgar,** vice president, and coordinator of Ethyl's various research activities, will assist Edward L. Shea, president, in technical matters and will continue as chairman of the research committee. Mr. Turner, who has been general manager of manufacturing, will be succeeded by **Clinton W. Bond.**

H. L. Turrittin, of the University of Minnesota, has been appointed to an associate professorship in mathematics at Princeton University.

Fletcher D. Woodward, professor of otolaryngology at the University of Virginia, retired as chairman of the School of Otolaryngology July 1, after 26 years of service, but will remain active on the teaching and clinical programs. **G. Slaughter Fitz-Hugh,** clinical assistant professor of otolaryngology, will succeed Dr. Woodward as chairman of the department.

Fumio Yamasaki, secretary of the Scientific and Technical Administration Committee of the government of Japan, recently visited the Bureau of Standards to study methods for the safe handling of radioactive materials and measurement of radioactivity. **G. Goudswaard,** director of the Permanent Office, International Statistical Institute, The Hague, also visited NBS.

Education

The **Fish and Wildlife Service** exploratory fishing vessel, *Western Explorer*, has started on a four-month bluefin tuna survey in New England waters, chiefly off the shores of Maine and Massachusetts. Primary objective is the development of a new fishery in the region. Cruise members will try to locate commercial concentrations of bluefin tuna, determine their pattern of abundance, direction of migration, and potential quantities available. John J. Murray is in charge of the investigation.

Florida State University has been authorized to confer the Ph.D. in meteorology, the only institution in the southeastern U. S. that offers such a professional program. Wouter Bleeker, on leave of absence from the University of Utrecht, has been named visiting professor of meteorology. Dr. Bleeker was recently elected first president of the Commission for Synoptic Meteorology of the World Meteorological Organization. Leon Sherman, research associate in the Institute of Geophysics at the University of California (Los Angeles), has been named assistant professor.

Howard University will offer a Thursday evening chemistry lecture series October 18–May 15, in which the following speakers will appear: H. C. Brown, P. L. Julian, H. F. Mark, G. E. Boyd, I. M. Koltoff, S. A. Waksman, C. D. Coryell, G. W. Wheland, M. Calvin, and P. J. W. Debye.

The Iowa State College–Guatemala Tropical Research Center at Antigua has invited R. H. Painter, Kansas State College entomologist, to Guatemala for six weeks to look for strains of corn resistant to several kinds of corn borer, the corn ear worm, and other insect pests.

New York University will offer a new program beginning September 24 in adult education (17 courses) at its University Heights Center in the Bronx. For further information, write to the Coordinator of the Institute of Adult Studies.

The *Horizon,* **Scripps Institution of Oceanography** research vessel, sailed this month for a two-month cruise to gather oceanographic information to fill in blank areas on the charts of the Pacific north of the San Francisco–Hawaii steamer lanes and south of the Aleutians. The cruise is sponsored by ONR in cooperation with the Navy Electronics Laboratory at San Diego. Attempts will be made to dredge samples from the Mendocino escarpment and from the tops of seamounts near Alaska, with continuous soundings of the bottom being made.

The University of Tennessee College of Medicine will open on September 27 a new clinic to be known as the Family General Practice Clinic, in the Out-Patient Department of John Gaston Hospital. General practitioners from the Memphis area will act as visiting physicians, and Paul Williamson, general practitioner of Vernal, Utah, will be director.

Grants and Fellowships

The **Althouse Chemical Company** has established a fund amounting to $4,000 at Lehigh University, to be used for graduate fellowship grants, purchase of equipment, or in any way beneficial to the field of organic chemistry.

The **American Society of Refrigerating Engineers** has authorized grants to Oklahoma A & M for research on refrigerant desiccants begun at Louisiana State under the direction of L. H. Bartlett, who has recently transferred to Oklahoma A & M. At Case Institute, W. L. Bryan will continue his work on direct expansion evaporators. At Saint Louis University, B. J. Luyet and K. O. Beatty will study preservation of frozen plant tissues and problems of heat transfer. The University of Kentucky has received additional funds to continue its work on the storage of meat.

Central Scientific Company has awarded scholarships for graduate study in the physical sciences to Edwin F. C. Cain at Michigan State and to Josef Anton Hofmann, who will work under N. F. Ramsey at Harvard.

The **Jane Coffin Childs Memorial Fund for Medical Research** has appropriated $254,587 in the period November–June for support of cancer research projects and fellowships in Denmark, England, Switzerland, and the U. S. The following were the recipients of aid: Tod W. Campbell, *Cancer Research*, William U. Gardner, Harry S. N. Greene, Alexander Haddow, Heinz Herrman, Henry D. Hoberman, Charles W. Hooker, Henry S. Kaplan, Niels O. Kjeldgaard, Harrison Latta, C. C. Little, Martin Lubin, Frank Lundquist, Basile J. Luyet, Leon L. Miller, John C. Sonne, L. C. Strong, Edward L. Tatum, John J. Trentin, and Yale University School of Medicine.

Commercial Solvents Corporation has given $12,000 to the Hektoen Institute for work on the viruses of *Borreliota* and *Miyagawanella*. A **Weissman Memorial Grant** is supporting the Salmonella Typing Center, which receives cultures chiefly from South America, the Congo, and India. A gift of $10,000 from the **Newberry Fund** enables the Division of Parasitology to maintain a type culture collection of blood flagellates for the Midwest Society of Parasitologists, and a grant from the **Rothschild Memorial Fund** has made it possible to extend the work of the cholera project.

The **Educational Testing Service** has appointed H. Paul Kelly, of the University of Texas, Samuel J. Messick, of the University of Pennsylvania, and Richard E. Wortman, of the University of Washington, as recipients of the annual fellowship awards for the Psychometric Training Program at Princeton. Robert P. Abelson and Miles S. Rogers were reappointed for the coming year.

NRC's Committee on Problems of Alcohol has made research grants totaling about $25,000 to M. X. Zarrow, Purdue; Curt P. Richter and A. Earl Walker, Johns Hopkins Medical School; Robert G. Grenell, University of Maryland; H. R. Hulpieu, Indiana University School of Medicine; and John C. Forbes, Medical College of Virginia. The committee is available for advice on the merits of specific projects in the field.

The **Fund for the Advancement of Education** has awarded 250 Faculty Fellowships (*Science*, 113, 541, 569 [1951]), amounting to $1,096,870 in addition to travel and tuition costs. Nearly 175 institutions are represented, as follows: Northeastern states, 52; North-Central, 38; Southern, 62; Western, 22.

Thirty-seven medical schools and 16 dental schools are the recipients of **Public Health Service** grants totaling $885,067 to improve instruction in cancer diagnosis and treatment. The only new grant was made to establish a cancer training program at the University of Puerto Rico Medical School.

Fifty-six scholarship awards, of which 29 were renewals, have been made by the **Shapiro Foundation** to undergraduate students in the New Jersey, New York, Massachusetts, and Pennsylvania areas. The awards have been made annually since 1934.

In the Laboratories

The **American Association of Candy Technologists** has appointed a committee to cooperate with the Committee on Chemicals in Foods of the Manufacturing Chemists' Association. C. R. Korekel, president of Korekel-Oetinger, of Philadelphia, is chairman; Justin Alikonis, Ernest C. Peakes, and Waldemar H. Haug are his associates.

The **Arnold Engineering Development Center** at Tullahoma, Tenn. (about 100 miles from Oak Ridge), was dedicated by President Truman late in June. Although under the supervision of the USAF, it will develop and test equipment for all government agencies. Work will focus on problems of supersonic and transonic speeds and jet propulsion, however.

American Cyanamid's **Calco Chemical Division** has named R. J. Turner chief chemist for pharmaceuticals. Dr. Turner entered the Pharmaceutical Department in 1944 as a research chemist.

The first sulfuric acid plant in the Philippines, being built by the newly organized **Chemical Industries of the Philippines**, will probably begin operation by the end of August. The plant represents an initial investment of ₱500,000 and will help to meet the need for sulfuric acid until completion of the Maria Cristina hydroelectric and fertilizer plant in 1953.

The $2,000,000 **Johnson & Johnson Research Center** started operations in North Brunswick Township, N. J., on June 21. William H. Lycan, director of research, announced that 75% of the work will be devoted to processing and improving present products, and 25% to fundamental research.

Naugatuck Chemical Division of the United States

Rubber Company will begin this month the construction of new buildings and equipment aimed at the doubling of production at its Baton Rouge, La., Paracril synthetic rubber plant. The first commercial GR-S produced in the U. S. was made at this plant in 1941, by technicians of Esso Standard Oil (former owners) and U. S. Rubber.

Oliver Iron Mining Company has started the construction of a pilot taconite beneficiation plant at Mountain Iron, Minn. The plant probably will be placed in operation during the summer of 1952.

Meetings and Elections

Otto R. Frisch, Jacksonian professor of natural philosophy at Cambridge University and head of the Nuclear Physics Section at the Cavendish Laboratory, has been added to the list of speakers in the symposium on **Nuclear Physics in Europe** (*Science*, **114**, 84 [1951]).

Talks of general interest at the Oak Ridge symposium on **The Role of Engineering in Nuclear Energy Development** will be given by T. Keith Glennan, Lawrence R. Hafstad, and C. G. Suits. Specialized aspects of the subject will be discussed by 19 other engineers, educators, and physicists from government and industrial laboratories. The symposium is under the sponsorship of the American Society for Engineering Education.

The **Society for the Advancement of Management** has elected Leon J. Dunn president and Edward W. Jochim executive vice president; Bruce Payne, treasurer; Howard K. Hyde, secretary; and Dillard E. Bird, retiring president, director-at-large.

Robert C. Swain, vice president in charge of research and development, American Cyanamid Company, has been elected honorary chairman of the American section of the **Society of Chemical Industry** for 1951–52. Other honorary officers elected were: Harry B. McClure, vice chairman; Cecil L. Brown, treasurer; Robert Heggie, controller; and Frederick W. Adams, secretary.

The **World Chemical Conclave** in New York September 3–13 will be attended by more than 200 young European chemists and chemical engineers, who would not otherwise have the means to visit the U. S., under the sponsorship of ECA and the Organization for European Economic Cooperation. A similar opportunity for 60 young chemists from Asia, Africa, South America, Australia, and New Zealand will be provided through a grant by the Ford Foundation. After the conclave the visiting chemists will spend three to four weeks touring chemical factories and government, university, and industrial laboratories in the U. S. Erwin Brand, of Columbia, is chairman of the special advisory committee of the American Chemical Society that is supervising the projects.

The fourth **World Health Assembly** elected Leonard A. Scheele, surgeon general of the USPHS, president for the coming year. New vice presidents are D. A. Dowling, of Australia, A. H. Taba, of Iran, and K. Evang, of Norway. Chairman of the program committee is M. Jafa, Pakistan director-general of health.

E. V. Murphree, president of Standard Oil Development Company, has been elected chairman of the permanent council of the **World Petroleum Congress.** Vice chairmen are G. A. Tuyl Schuitemaker, M. S. Scheer, C. P. Southwell, and Carlos Perez de la Cova. The fifth congress will be held in the U. S. in 1959, coinciding with the 100th anniversary of the first drilling for oil in Titusville, Pa.

Miscellaneous

The 36th edition of ***The Naturalists' Directory*** has just been published at Salem, Mass. It contains the names, addresses, and special subjects of study of professional and amateur naturalists of North and South America and a list of scientific periodicals and natural history museums.

Chemicals wanted by Armour Research Foundation of Illinois Institute of Technology, Registry of Rare Chemicals, 35 W. 33rd St., Chicago 16, are: 2,3,4-trimethylhydroxybenzene; 2,4,6-trimethylhydroxybenzene; 2,4,6-trichlorotoluene; 2,4,6-trichlorostyrene; tetraethyleneglycol dimethyl ether; ortho-tosyl-*para*-hydroxybenzene sulfonic acid; propylene sulfide; propyleneimine; 2-nitro-4,5-dimethylaniline; hexacosane; stannous phosphide; copper thiocholine; trisilane; copper selenide; aleuronate; gymnemic acid; liquiritin; lithospermum ruderale; glycerylphosphorylcholine; and ovalene.

The **American Forestry Association** has arranged U. S. study tours for some 80 executives and technicians from eleven wood-using countries of Western Europe. The first group of 37 left on July 13, shortly before the scheduled arrival of the second group. The project is under the direction of George A. Garratt, of Yale, and is sponsored by the OEEC and ECA.

The **National Society for Medical Research** has awarded a Certificate of Merit to the Baltimore *Sun* for the excellence of its reporting of developments in the medical sciences. William R. Manchester, of the *Sun* staff, was also awarded a certificate for his feature articles on antivivisection. A third award was made to the Pitman-Moore Company, of Indianapolis, for a national advertising series on the role of animal research in major medical discoveries.

A Nutrition Bibliography, consisting of selected scientific literature pertaining to nutrition and to the nutritive value of foods in the Pacific islands, is being prepared by Carey D. Miller, of the University of Hawaii, at the request of the Seventh Pacific Science Congress.

Reeves Soundcraft Corp., of New York, has purchased the **Bergen Wire Rope Company,** of Lodi, N. J. and will operate it as a wholly owned subsidiary.